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TABLE OF CONTENTS

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TABLE OF CONTENTS---Continued

THE EFFECTS OF IONIZING RADIATIONS ON THE BIOCHEMISTRY OF MAMMALIAN TISSUES

I. Influence of X-Irradiation on the Development of a Thiophosphate-Oxidizing Enzyme System in the Livers of Young Male Rats

Bernard E. Hietbrink, Marjorie Keshmiri, and Kenneth P. DuBois

This report concerns: Results of additional experiments undertaken to obtain information concerning the influence of ionizing radiations on the development of the phosphorothioate-oxidizing enzyme in the livers of young male rats. The influence of 100 r and 200 r of x-ray, given at seven and fourteen-day intervals, on the synthesis of this enzyme system was investigated. Observations were also made on the influence of exogenous adrenal cortical extract on the development of the drug metabolizing enzymes and further studies were performed to ascertain the ability of 2-mercaptoethylamine (MEA) to prevent the inhibitory effect of radiation on the synthesis of this enzyme system.

Immediate or ultimate application of the results: The present investigation constitutes a continuation of studies on the effects of ionizing radiations on the development of microsomal enzymes in the liver which are responsible for the oxidative desulfuration of phosphorothioates and which catalyze the metabolism of other drugs and toxic compounds. During the course of this study we have attempted to obtain information concerning the influence of radiation on the mechanisms responsible for the normal development of these microsomal enzymes in young male rats. Results of previous studies have shown that single doses of 100 r or 200 r of x-irradiation cause substantial inhibition in the rate of development of the enzymes in young rats for approximately four weeks following exposure. The enzyme activity develops rapidly after this period and reaches normal adult levels at six weeks after 100 r or 200 r of x-ray. Data has been obtained indicating that the inhibition of the development of the enzyme activity may be due to damage to systems other than those found in the liver or to the ability of shielded tissues to supply some substance essential for development of microsome enzymes in the liver. It was also found that the inhibition caused by 200 r or 400 r of x-ray can be significantly reduced by the administration of MEA prior to x-ray exposure. The present investigations, which are a continuation of these studies, show that the administration of a second dose of 100 r or 200 r of x-ray seven days after the initial exposure prolongs the inhibitory effect of radiation on the development of the microsome enzymes. However, giving a second radiation exposure fourteen days after the first exposure does not substantially influence the amount of delay in enzyme synthesis. Evidence is presented that administration of MEA before 500 r of x-irradiation does not prevent the inhibitory effect on the development of enzyme activity. It is anticipated that the information obtained in these experiments will provide information that will be useful in ascertaining the mechanisms involved in the injurious action of ionizing radiations on mammalian tissues.

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Recent studies in this laboratory indicated that sublethal doses of x-irradiation have a marked inhibitory influence on the development of microsome enzymes that are necessary for the metabolism of various drugs and toxic agents (1) in the livers of young male rats. A systematic study was undertaken to obtain information concerning the radiation-induced defect in the synthesis of microsome enzymes. One phase of this study consisted of measurements of the influence of x-ray on the development of a microsome oxidase which catalyzes the desulfuration of the phosphorothioate, dimethyl-2-(4-oxo-1,2,3-benzotriazinyl-3-methyl) phosphorodithioate (guthion, DBD), to its oxygen analogue.

Results of these studies have indicated that doses of x-ray as low as 100 r (2,3) cause substantial reduction in the rate of development of the enzyme system, that the inhibition is reversible, and that the enzyme activity reaches normal adult levels at five to six weeks after 100 r or 200 r of x-irradiation (4). Experiments undertaken to gain information on the gross site of radiation-induced inhibition showed that shielding of the testes or of the liver area did not prevent the inhibitory effect of 200 r or 400 r of x-ray on the activity of this enzyme (3). Recently it was found that the administration of 600 r of x-ray to the liver area while shielding the remainder of the body reduced the rate of development of the enzymes during the latter part of the observation period (3). The present report describes results of additional experiments on the influence of x-irradiation to the liver area on the synthesis of this enzyme system.

Measurements were made of the ability of a radioprotective compound to prevent the inhibitory effect of radiation on the development of the phosphorothioate-oxidizing enzyme. Administration of 200 mgm./kgm. of MEA ten minutes before 200 r or 400 r of x-ray reduced the degree of inhibition of enzyme synthesis caused by x-irradiation (5). The results of further studies concerning the ability of MEA to reduce the radiation-induced synthesis of the liver oxidase system are described in this report. Data concerning the influence of repeated exposures of x-irradiation and daily injections of adrenal cortex extract on the development of microsome enzyme activity are also included in this report.

Materials and Methods. Young, male Sprague-Dawley rats were used for these experiments. The animals were housed in air-conditioned quarters and were given Rockland Rat Diet and water ad libitum. X-irradiation was administered as a single exposure with a G. E. Maximar therapy unit employing the following radiation factors: 250 KVP, 15 ma., 0.25 mm. Cu and 1 mm. Al added filtration. The target-animal distance was 75 cm. giving a dose rate of 34 r to 36 r per minute as measured in air with a Victoreen ionization chamber. For experiments on the effect of partial body shielding on the development of the drug metabolizing enzyme system, weanling rats (23 days old) were anesthetized with aqueous solutions of sodium pentobarbital (25 mgm./kgm. intraperitoneally) to facilitate accurate placement and maintenance of the lead shields during radiation exposure. Neutral aqueous solutions of MEA were freshly prepared and injected intraperitoneally ten minutes before administration of x-irradiation. Sterile solutions of Adrenal Cortex Injection U.S.P. were given daily by the intramuscular route.

For enzyme assays the rats were sacrificed by decapitation and the livers were quickly removed, weighed and homogenized in cold distilled water. Guthion was converted to its active metabolite by the method developed by Murphy and DuBois (6) in this laboratory and by a modification of the method used by Conney et al. (7) for other reactions catalyzed by microsome enzymes. The details of these modifications and the methods employed in the calculation of the enzyme activity have been described in a previous report from this laboratory (5).

Results

Influence of repeated doses of 100 r or 200 r of x-irradiation on the development of the phosphorothioate-oxidizing enzyme system in the livers of young rats. Previous studies in this laboratory have shown that doses of radiation ranging from 100 r to 400 r cause a substantial inhibition of the development of the enzyme system in the livers of young male rats which is responsible for the oxidative desulfuration of phosphorothioates (2,3). The radiation-induced inhibition of the development of this enzyme system is reversible and the enzyme activity reaches normal adult levels at four to six weeks after 100 r or 200 r of x-ray (4). In order to obtain information on the influence of repeated exposures of x-irradiation on the development of enzyme activity, groups of animals which had received 100 r of x-ray at 23 days of age were given another dose of 100 r at 30 days or 37 days of age and groups which had been exposed to 200 r of radiation at 23 days were given 200 r at 30 days or 37 days of age. The animals were sacrificed at various intervals during the following three weeks, a portion of the liver was removed and the activity of the microsome enzymes was measured. The results of these measurements are shown in Figures 1 and 2 where each point on the curves is the average of measurements on the livers of at least four animals.

The data in Figure 1 show that the livers of animals which were given 100 r of x-ray at 23 days and 100 r at 37 days of age exhibited a rate of development of microsome enzyme activity which was similar to that observed in the liver of rats that had been given this dose of radiation at 23 days of age, however, the enzyme activity of the livers of rats which were given a second exposure of 100 r at 30 days of age was substantially inhibited until the animals reached 60 days of age. The data in Figure 2 illustrate that repeated exposures to 200 r produce an effect similar to that shown in Figure 1. The enzyme activity of the livers of rats which were given a second dose of 200 r of x-ray at 37 days of age reached an adult level at approximately the same rate as the animals which were given 200 r of x-irradiation at 23 days of age. The administration of a second dose of 200 r of x-ray at 30 days of age prolonged the radiation-induced inhibition of the synthesis of the drug metabolizing enzyme system.

Influence of 2-mercaptoethylamine on the radiation-induced inhibition of the development of the phosphorothioate-oxidizing enzyme system in the livers of young male rats. The results of studies presented in our previous report (5) showed that 200 mgm./kgm. of MEA reduced the degree of inhibition of the development of the phosphorothioate-oxidizing enzyme caused by 200 r or 400 r of x-irradiation. It was observed that 400 r of x-ray caused a delay in the development of the enzyme system in the MEA-treated rats and it appeared unlikely

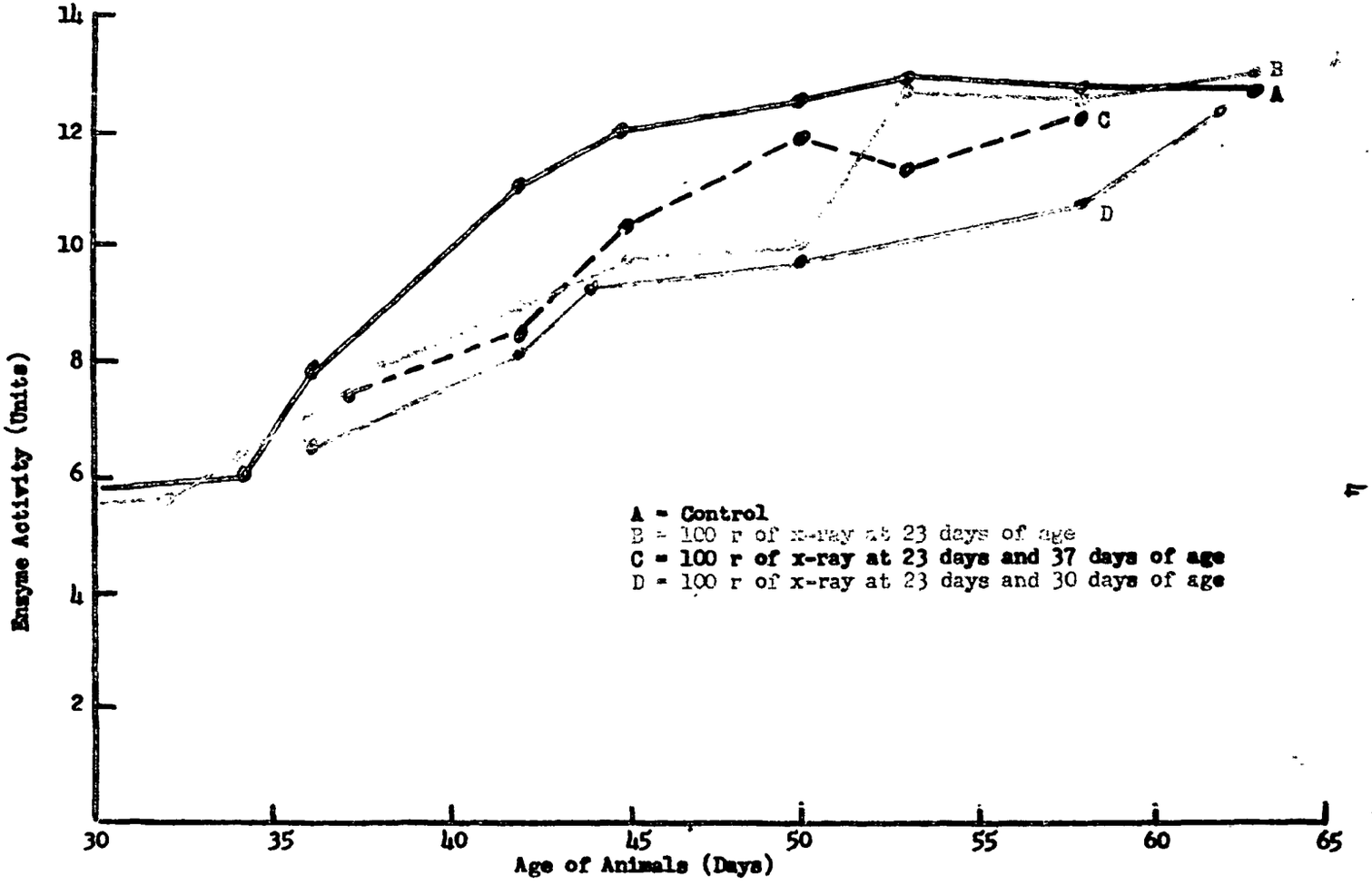

Figure 1. Influence of repeated doses of 100 r of x-irradiation on the development of the phosphorothioate-oxidising enzyme system in the livers of young male rats.

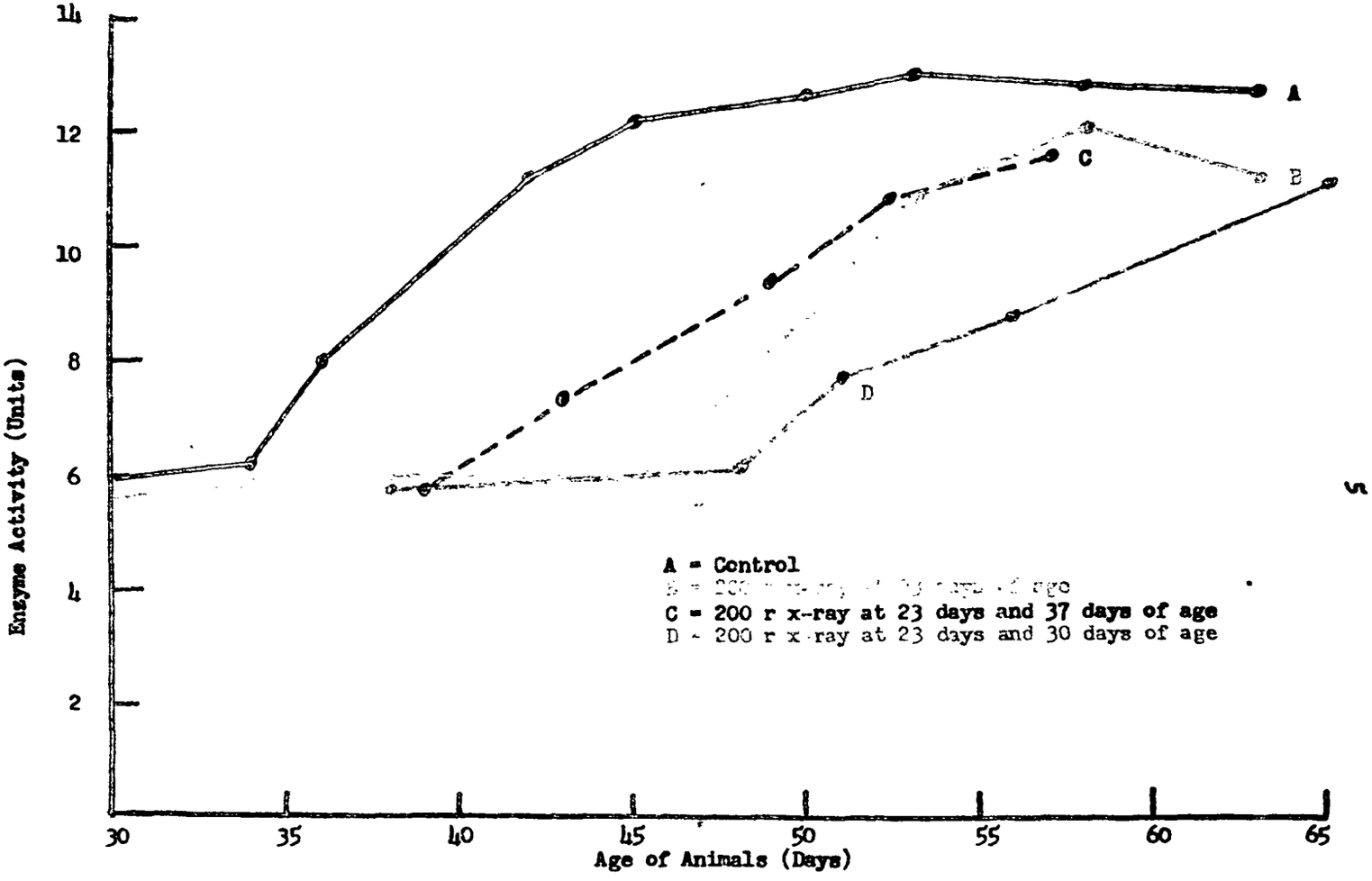

Figure 2. Influence of repeated doses of 200 r of x-irradiation on the development of the phosphorothioate-oxidizing enzyme system in the livers of young male rats.

that the radioprotective activity of this compound would be sufficient to prevent the inhibitory effect of higher doses of x-irradiation. To obtain additional information on the ability of MEA to reduce the radiation-induced inhibition of the development of the drug metabolizing enzymes in the liver, 23-day old male rats were given intraperitoneal injections of 200 mgm./kgm. of MEA ten minutes before 500 r of x-irradiation. The animals were sacrificed and a portion of the liver removed for enzyme measurements at frequent intervals for a period of three weeks after x-irradiation. The results of these measurements are presented in Figure 3 where each point on the curves for the irradiated animals is the average of measurements on the livers of at least four animals and each point on the control curve is the average for 8 to 10 animals.

The data in Figure 3 show that 200 mgm./kgm. of MEA given ten minutes before 500 r failed to prevent the radiation-induced inhibition of the development of the phosphorothioate-oxidizing enzyme system in the livers of young male rats. Thus it is evident that MEA is not capable of preventing the inhibitory effect on the development of the drug metabolizing enzyme caused by doses of x-irradiation in excess of 400 r.

Influence of partial body shielding on the development of the phosphorothioate-oxidizing enzyme system in the livers of young male rats. The results of previous studies have shown that shielding the testes or the liver area does not prevent the inhibitory effect of 200 r of x-irradiation on the development of the phosphorothioate-oxidizing enzyme system in the liver of young male rats (2,3). These experiments also demonstrated that when the liver area was exposed to 200 r or 400 r of x-ray with the remainder of the body shielded synthesis of the drug metabolizing enzyme system in the liver was not inhibited (3,4). More recent observations have shown that 600 r of x-ray to the liver area caused a substantial inhibition in the rate of development of the phosphorothioate-oxidizing enzymes in the liver during the 12 to 21-day period following 600 r of x-irradiation. The present study was undertaken to determine the influence of higher doses of x-irradiation on the development of the enzyme activity in the liver. For these experiments 23-day old male rats were anesthetized with 25 mgm./kgm. of sodium pentobarbital and lead shields were placed so as to shield the entire body except the liver area. This area was then given 600 r of x-irradiation. The animals were sacrificed at various intervals during the following three weeks, a portion of the liver was removed and the microsome oxidase activity was measured. The results of these measurements are presented in Figure 4 where each point on the curves is the average of measurements on the livers of at least four animals.

The influence of 200 r of whole body x-irradiation and of 600 r of x-ray to the liver area on the synthesis of the drug metabolizing enzyme in the livers of male rats has been presented in previous reports and is included in Figure 4 for purposes of comparison. The data illustrate that 800 r of x-ray administered to the liver area, like 600 r to this area, causes a substantial inhibition in the rate of synthesis of the enzyme activity during the 12 to 21-day period following x-irradiation.

Influence of daily injections of adrenal cortex extract on the radiation-induced inhibition of the development of the phosphorothioate-oxidizing enzyme system in the livers of young male rats. Results of recent studies on the influence of partial body shielding have indicated that shielding the liver area while

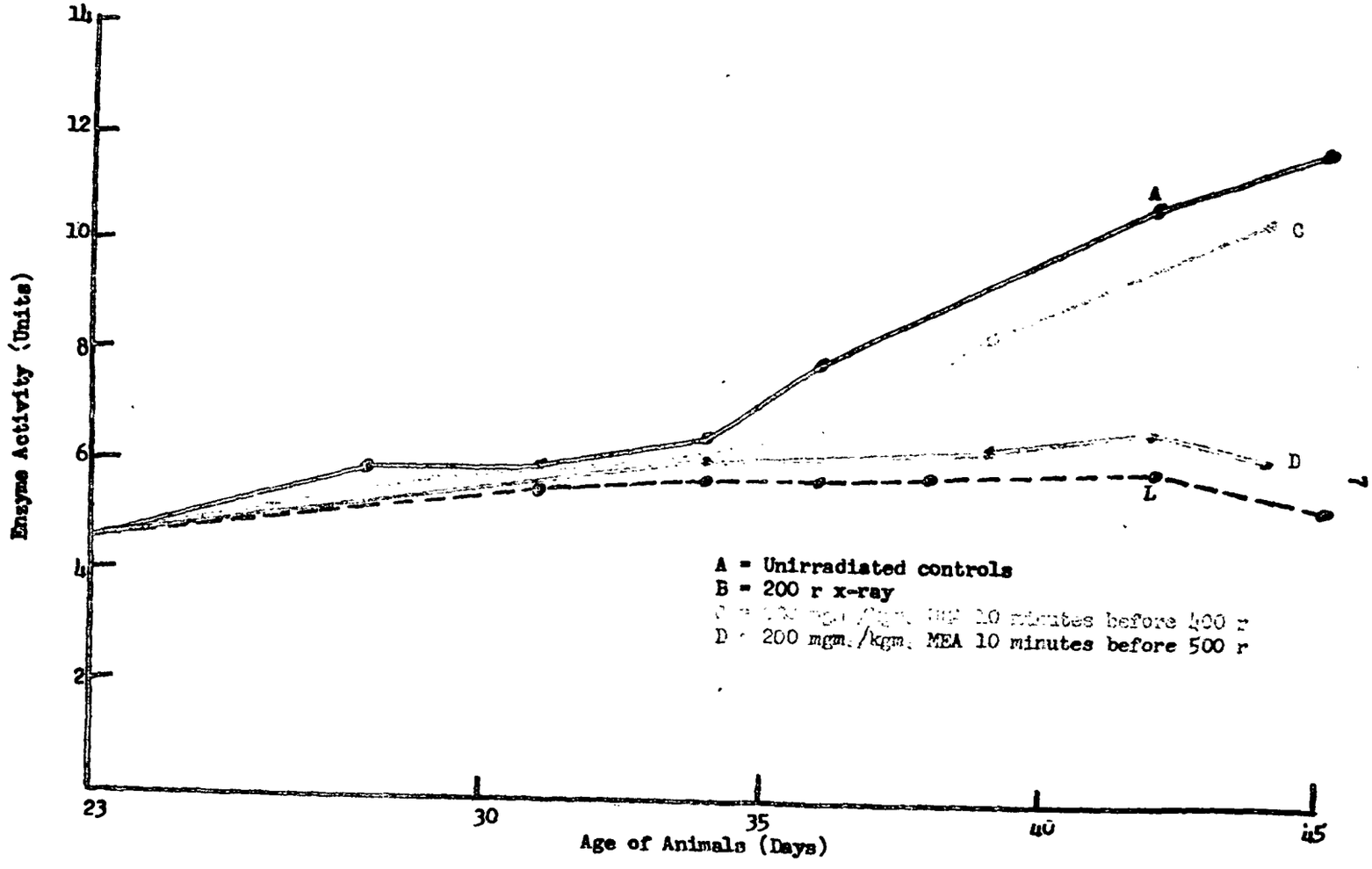

Figure 3. Influence of 2-mercaptoethylamine on the radiation-induced inhibition of the development of the phosphorothioate-oxidizing enzyme system in the livers of young male rats.

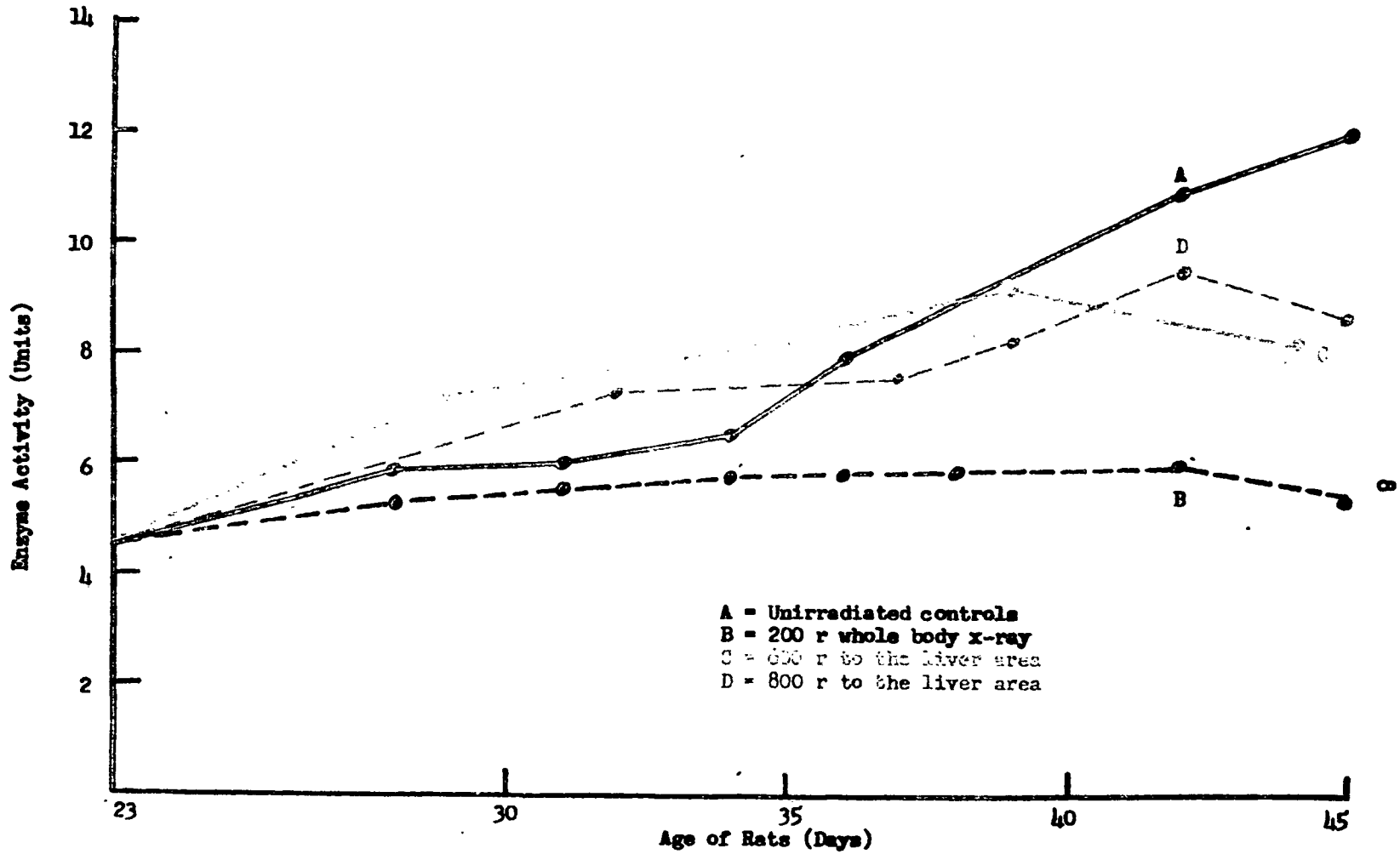

Figure 4. Influence of partial body shielding on the development of the phosphorothioate-oxidizing enzyme system in the livers of young male rats.

exposing the remainder of the body to 200 r of x-ray reduced the inhibitory effect of radiation on the development of the phosphorothioate oxidizing enzyme (3), that shielding the testes did not affect the inhibitory action of 200 r (2) and that 600 r and 800 r of x-ray administered to the liver area caused a significant degree of inhibition in the synthesis of this enzyme system. In view of these results it seemed possible that radiation damage to the adrenal glands might play a role in the delay in the development of the drug metabolizing enzyme. Thus studies were undertaken to obtain information concerning the influence of adrenal cortex extract on the radiation-induced inhibitory effect on the synthesis of microsome oxidases. For these experiments weanling rats were given 200 r of whole-body x-irradiation and 0.1 ml./day of adrenal cortex extract was administered intramuscularly. The animals were sacrificed at various intervals during the following 3-week period and the microsome oxidase activity of the liver was measured. The results of these experiments, which are shown in Figure 5, indicate that daily injection of adrenal cortex extract reduced the degree of radiation-induced inhibition of the development of the phosphorothioate-oxidizing enzyme caused by 200 r. These results provide evidence which indicates that radiation-induced injury to the adrenal glands may be a factor in the delayed development of enzyme activity in the livers of young male rats. Additional studies, designed to obtain more conclusive evidence concerning the role of adrenal cortical hormones in the synthesis of this enzyme system are currently in progress.

Discussion

The present investigation was undertaken to obtain additional information concerning the influence of ionizing radiations on the development of the enzymes in the livers of young male rats which are responsible for the metabolism of various drugs and toxic agents. The present report concerns a study of the influence of repeated doses of 100 r or 200 r of x-irradiation at 7 days and 14 days after the initial exposure on the synthesis of the phosphorothioate-oxidizing enzymes, a continuation of previous studies to determine the effect of partial body shielding or pretreatment with 200 mgm./kgm. of MEA on the inhibitory effect of x-ray on the microsome oxidases as well as the results of initial experiments designed to obtain information on the influence of adrenal cortical hormones on the development of these enzymes. The results of these studies indicated that the administration of a second dose of x-irradiation to 30-day old rats at seven days after the initial exposure increased the period required for synthesis of enzyme activity; however, when the second dose was given 14 days after the first exposure the microsome oxidases developed at a rate similar to animals irradiated only at 23 days of age. It was found that the administration of 800 r of x-ray to the liver area did not inhibit the initial development of the enzymes responsible for drug metabolism but that the synthesis of the system was substantially inhibited during the latter portion of the observation period. It was noted in this and previous experiments (3,4,5) that radiation appeared to enhance the development of the phosphorothioate-oxidizing enzymes in the liver for approximately 10 to 12 days following x-ray. The exact mechanism responsible for this effect is not readily apparent, however, it seems likely that sodium pentobarbital used to immobilize the animals during the shielding experiments may be causing this initial stimulation in activity since Conney et al. (8) have shown that relatively small doses of other barbiturates cause marked increases in the activity of various drug-metabolizing enzymes which are located in the liver microsomes.

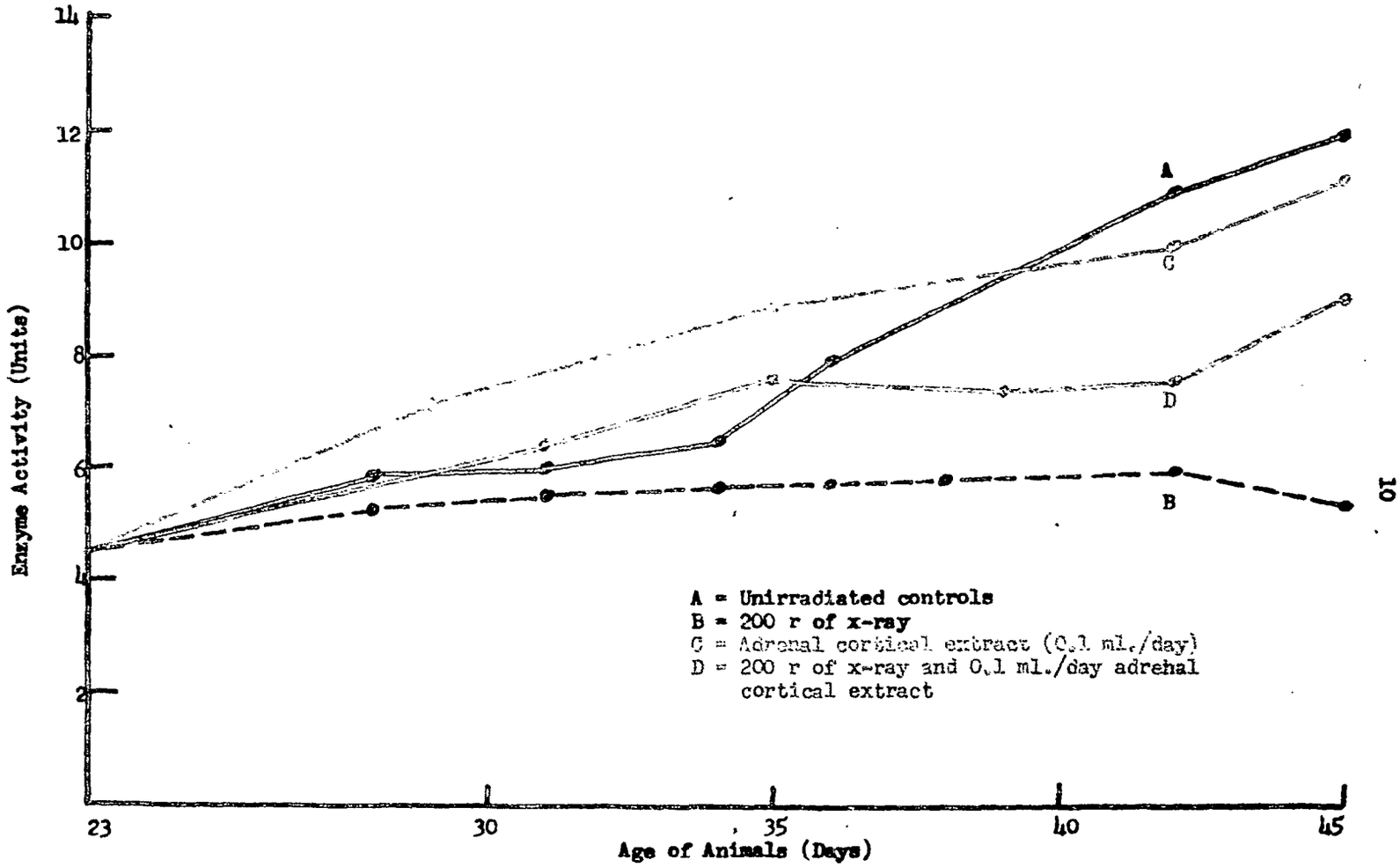

Figure 5. Influence of daily injections of adrenal cortex extract on the radiation-induced inhibition of the development of the phosphorothioate-oxidizing enzyme system in the livers of young male rats.

Further studies are currently in progress to obtain information concerning the influence of x-irradiation on the increases in phosphorothioate-oxidizing enzyme activity caused by barbiturates and other chemical compounds.

The results of preliminary studies on the influence of adrenal cortical hormones on the radiation-induced inhibition of the development of the drug metabolizing enzymes showed that daily injections of 0.1 ml. of adrenal cortex extract reduced the degree of inhibition caused by 200 r of x-ray. These results provide evidence that radiation-induced inhibition of the development of microsome enzyme activity may be partially due to an insufficient release of adrenal cortical hormones. Additional studies designed to provide information on the effect of exogenous adrenal cortical hormones on development of the phosphorothioate-oxidizing enzyme activity in partially shielded and total body irradiated animals are currently in progress.

Summary

1. Studies were undertaken to determine the influence of repeated exposures of 100 r or 200 r of x-irradiation on the development of the phosphorothioate-oxidizing enzyme system in the livers of young male rats. It was found that the enzyme activity of the livers of rats which were given 100 r or 200 r of x-ray at 23 days of age and a like dose at 37 days of age reached the normal adult level at approximately the same time as the livers of animals which were irradiated at 23 days. Administration of the second dose of 100 r or 200 r of x-ray at 30 days of age increased the period of radiation-induced inhibition of development of enzyme activity.

2. The injection of 200 mgm./kgm. of MEA ten minutes before 500 r of x-ray failed to substantially reduce the radiation-induced inhibition of the synthesis of the drug metabolizing enzyme system in the livers of young male rats.

3. The results of experiments on the influence of partial body shielding indicated that the administration of 800 r of x-irradiation to the liver area of 23-day old rats caused a substantial inhibition in the rate of synthesis during the 12 to 21-day period following radiation.

4. Measurements of the influence of daily intramuscular injections of adrenal cortex extract on the radiation-induced inhibition of the development of the phosphorothioate-oxidizing enzyme system were performed. Results of these measurements indicated that daily injections of adrenal cortex extract reduced the degree of radiation-induced inhibition in the synthesis of the drug metabolizing enzymes in the livers of young male rats.

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THE EFFECTS OF IONIZING RADIATIONS ON THE BIOCHEMISTRY OF MAMMALIAN TISSUES

II. The Influence of Various Chemical Compounds on Radiation-Induced Changes in Enzyme Activities in Certain Rat Tissues

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Mary E. Hayward

This report concerns: The results of studies on the influence of chemical compounds on the injurious effects of x-irradiation. The present study has been concerned with the quantitative measurements of the radioprotective activity of 2-imino-thiazolidine-4-carboxylic acid and of the influence of oral administration of various combinations of cysteine, 2-aminoethylisothiouronium (AET) and 2-mercaptoethylamine (MEA) on the radiation-induced changes in enzyme activity of the spleens, thymus glands and small intestines of the rat. The results of 30-day survival studies on animals treated with 2-imino-thiazolidine-4-carboxylic acid before and after lethal doses of x-irradiation are also included.

Immediate or ultimate application of the results: To obtain information concerning the ability of various chemical compounds and mixtures of these agents to reduce the damaging effects of radiation on the spleens, thymus glands and intestines of rats. Results of a previous study (1) on the influence of oral administration of radioprotective compounds indicated that the maximum protective effect of MEA by this route occurred at two hours after administration. However, very little information is available concerning the ability of radioprotective agents to prevent radiation injury when given orally. Data of this type would be of considerable value in determining the practical use of chemical compounds as radioprotective agents. Recent studies by Melville et al. (2) have shown that the oral administration of mixtures of cysteine and AET provides significant protection against the lethal effects of x-irradiation in monkeys. The current study was undertaken to determine the radioprotective activity of 2-imino-thiazolidine-4-carboxylic acid, a compound not previously tested for protective activity in rats, and to obtain additional information concerning the influence of the oral administration of mixtures of certain sulfur-containing compounds on the radiation-induced changes in the enzyme activities of the spleen, thymus glands and small intestine. It is hoped that these studies may lead to the formulation of a drug treatment that will provide optimum radioprotection when given by the oral route.

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Recent studies in this laboratory (3) showed that 2-imino-thiazolidine-4-carboxylic acid, a minor metabolite formed in the detoxification of cyanide, protected mice against the lethal effects of x-irradiation. This agent is of particular interest because of its structural similarity to AET. In our

previous study (4) it was found that the intraperitoneal administration of 125 mgm./kgm. of this compound 25 minutes before x-ray provided substantial reduction in the biological effect of 400 r in the spleen. In view of these results additional studies were undertaken to determine the influence of various doses of this thiazolidine derivative on the radiation-induced changes in the enzyme activity of certain tissues of the rat and to ascertain the ability of this compound to prevent mortality caused by 850 r of x-irradiation. The results of these studies indicated that 140 mgm./kgm. of the thiazolidine derivative given 10 minutes before 400 r reduced the degree of change in the enzyme activity of the spleens and small intestines and permitted 60% survival of rats exposed to a lethal dose of 850 r.

Results of our recent studies (1,5) on the influence of oral administration of various radioprotective agents on the radiation-induced changes in the adenosine triphosphatase activity of the hematopoietic tissues and of the cholinesterase activity of the small intestine indicated that in general the compounds were more effective when administered intraperitoneally. Melville et al. (6) have found that the administration of a mixture of AET and cysteine provided a similar degree of radioprotection in monkeys when given by the oral or intravenous routes. Thus it was of interest to study the radioprotective effects of mixtures of some sulfur-containing agents given orally to rats. Results of this study indicated that the mixture of 1,000 mgm./kgm. of cysteine and 600 mgm./kgm. of AET provided substantial protection to all the tissues studied when given orally 30 minutes before 400 r.

Materials and Methods. Adult, female Sprague-Dawley rats were used for these experiments. The animals were housed in air-conditioned quarters at 68° to 75° F. and were given Rockland Rat Diet and water ad libitum. X-irradiation was administered as a single whole body exposure with a G. E. Maximar Therapy unit employing the following radiation factors: 250 KVP, 15 ma., 0.25 mm. Cu and 1 mm. Al added filtration. The target-animal distance was 75 cm. giving a dose rate of 34 r to 36 r per minute as measured in air with a Victoreen ionization chamber. The procedure employed for the synthesis of 2-imino-thiazolidine-4-carboxylic acid, which was prepared in this laboratory, is presented in detail in a previous report (3). The other compounds tested for radioprotective activity were injected as neutral aqueous mixtures. In all cases the concentrations were adjusted to permit injections of total volumes not exceeding 1% of the body weight.

The adenosine triphosphatase activity of the spleens and thymus glands was measured according to the method of DuBois and Potter (7) using 0.5% homogenates of spleen and 1% homogenates of thymus glands. Assays were performed in duplicate using 0.1 ml. and 0.2 ml. of each aqueous tissue homogenate. Inorganic phosphorus was determined by the method of Fiske and Subbarow (8) and the enzyme activity was expressed as micrograms of phosphorus liberated from adenosine triphosphate by 1 mgm. of tissue during a 15-minute incubation period. The acetylcholinesterase activity of the small intestine was determined by the manometric method of DuBois and Mangun (9). A portion of the small intestine was freed from the mesenteric connective tissue and fat and longitudinally dissected to expel the contents. The tissue was washed with distilled water, blotted with filter paper, minced and homogenized in Ringer-bicarbonate buffer. Measurements were conducted in duplicate using 50 mgm. of tissue per Warburg vessel. The vessels were gassed with 5% CO_2 and 95% N_2

for five minutes. Carbon dioxide evolution was recorded at 5-minute intervals for a period of 30 minutes following a preliminary 10-minute equilibration. Acetylcholinesterase activity was expressed as microliters of CO_2 evolved per 50 mgm. of tissue during a 10-minute incubation period. The degree of radioprotection provided by the chemical compounds in the tissues studied was expressed as per cent reduction of the biologically effective radiation dose. The data presented in this report were calculated using the dose response curves and methods described in a previous report (10).

Results

The effects of 2-imino-thiazolidine-4-carboxylic acid and 2-mercaptoethylamine on the changes in enzyme activities of the spleens, thymus glands and intestines of rats three days after 400 r of x-irradiation. Results of experiments presented in our previous report (4) illustrated that 2-iminothiazolidine-4-carboxylic acid, a metabolite formed during the detoxification of cyanide, is capable of reducing the degree of radiation-induced change in the adenosine triphosphatase activity of the spleen. These studies indicated that the compound is relatively non-toxic to rats. Thus it was of interest to obtain information concerning the ability of higher doses of this compound to reduce the damaging effects of radiation in the hematopoietic tissues and intestines of rats. For these experiments groups each containing four rats were given 140 mgm./kgm. or 175 mgm./kgm. of the thiazolidine (expressed as equivalents of HCN) at 10 minutes or 25 minutes before 400 r of x-ray. Three days later the animals were sacrificed for adenosine triphosphatase assays on the spleens and thymus glands and for cholinesterase measurements on the small intestines. The results of these experiments are presented in Table 1.

The data presented in Table 1 show that 140 mgm./kgm. of the thiazolidine derivative given ten minutes before 400 r of x-irradiation provided a significant reduction in the biological effect of 400 r in the spleen and small intestine but did not benefit the thymus glands. Administration of 140 mgm./kgm. of the thiazolidine 25 minutes before 400 r of x-ray caused a 29% reduction in the effect of radiation in the spleen but did not reduce the damaging effect of radiation in the thymus glands and intestine. Increasing the dose of the thiazolidine to 175 mgm./kgm. failed to protect the spleen to the extent provided by the lower doses of this compound (4). No gross toxic manifestations were observed following the injection of the thiazolidine derivative. Therefore, it appeared to be a suitable agent to be given in combination with other radioprotective agents. MEA has been shown to be one of the most effective radioprotective agents in rats and it has been found to enhance the radioprotective activity of other sulfur-containing compounds. The data presented in Table 1 indicate that the administration of 140 mgm./kgm. of the thiazolidine in combination with 200 mgm./kgm. of MEA failed to protect the tissues studied to the extent of MEA given alone.

The effects of oral administration of the combination of cysteine and MEA or cysteine and AET on the changes in enzyme activities of the spleens, thymus glands and intestines of rats three days after 400 r of x-irradiation. Previous studies in this laboratory on the effect of oral administration of radioprotective compounds to rats have generally indicated that most radioprotective compounds are more effective when given intraperitoneally (5). In this connection it was found that the maximum protective effect of MEA by the oral

TABLE I

THE EFFECTS OF 2-IMINO-THIAZOLIDINE-4-CARBOXYLIC ACID AND 2-MERCAPTOETHYLAMINE ON THE CHANGES IN ENZYME ACTIVITIES OF THE SPLEENS, THYMUS GLANDS AND INTESTINES OF RATS THREE DAYS AFTER 400 r OF X-IRRADIATION

Treatment	Dose in mgm./kgm.	Time of Administration Before X-ray (Minutes)	Spleen ATPase[a]		Thymus Glands ATPase[a]		Intestinal Cholinesterase[b]	
			400 r Activity	Effective % Dose Reduction	400 r Activity	Effective % Dose Reduction	400 r Activity	Effective % Dose Reduction
None	...	..	51.4±3.0	..	20.2±1.1	..	69± 9	..
2-Imino-thiazolidine-4-carboxylic acid	140[c]	10	47.0±1.9	19	20.7±1.0	0	80± 7	16
2-Imino-thiazolidine-4-carboxylic acid	140[c]	25	45.7±2.5	29	20.0±1.1	0	69± 4	0
2-Imino-thiazolidine-4-carboxylic acid	175[c]	25	48.1±1.2	17	21.0±0.7	0	59± 3	0
2-Mercaptoethylamine	200	10	43.9±2.5	36	18.1±1.4	17	96±21	35
2-Imino-thiazolidine-4-carboxylic acid plus 2-mercapto-ethylamine	140[c] 200	25 10	48.8±2.2	13	18.4±1.0	15	85± 8	23

[a] Activity expressed as mgm. of P liberated from ATP/mgm. tissue/15 minutes.

[b] Activity expressed as µl. of CO_2 evolved/50 mgm. tissue/10 minutes.

[c] Expressed as mgm./kgm. equivalents of HCN.

route occurred at two hours after administration (1). Results of studies on the influence of oral administration of sodium diethyldithiocarbamate and dimethylammonium dimethyldithiocarbamate indicated that these agents were not capable of reducing the radiation-induced changes in adenosine triphosphatase activity of the spleen when given by this route (5). This study showed that the oral administration of p-aminopropiophenone (PAPP) provided substantial protection to the spleen and intestine. Melville (11) has recently found that the combination of AET and cysteine, given orally, provides marked protection against the lethal effects of x-irradiation in monkeys. Thus it was of interest to determine the radioprotective activity of certain combinations of AET, MEA and cysteine in rats. For these experiments groups each containing four rats were given various mixtures of cysteine and MEA or cysteine and AET 30 minutes before 400 r of x-irradiation. Three days later the animals were sacrificed for adenosine triphosphatase assays on the spleens and thymus glands and cholinesterase measurements on the intestines. The results of these measurements are presented in Table 2.

The data presented in Table 2 indicate that oral administration of the mixture of 1,000 mgm./kgm. of cysteine and 200 mgm./kgm. of MEA provided substantial protection to the hematopoietic tissues but did not benefit the intestine when given 30 minutes before 400 r of x-ray. Increasing the amount of MEA in the mixture to 250 mgm./kgm. did not improve the radioprotective qualities of this combination. Several dosage levels of AET were given in combination with 1,000 mgm./kgm. of cysteine. The most effective mixture of these compounds tested contained 600 mgm./kgm. of AET. This combination provided substantial reductions in the biological effect of x-ray in all the tissues studied.

The effect of 2-imino-thiazolidine-4-carboxylic acid on the 30-day survival of rats after 850 r of total-body x-irradiation. Results of experiments presented in this and our previous report (4) indicate that in certain instances 2-imino-thiazolidine-4-carboxylic acid provided significant reductions in the biological effect of 400 r in the tissues under investigation. In order to relate this protective effect to the ultimate survival of the animals 140 mgm./kgm. of the thiazolidine derivative was given at 25 minutes before, 10 minutes before, or 25 minutes after exposure of rats to a lethal dose of x-irradiation. The data presented in Table 3 show the effects of this compound on the 30-day survival and median survival time of animals treated in this manner. A dose of 850 r of x-irradiation caused 100% mortality and resulted in a median survival time of nine days.

The administration of 140 mgm./kgm. of 2-imino-thiazolidine-4-carboxylic acid ten minutes before x-ray permitted survival of 60% of the rats given 850 r. The injection of thiazolidine 25 minutes before x-ray was not as effective and prevented mortality in one of the five animals given 850 r of x-irradiation. Results of recent studies in this laboratory (3) indicate that this thiazolidine increased the median survival time and permitted significant survival of mice after 650 r of whole-body x-ray. Thus it was of interest to determine the radioprotective activity of this compound when given 25 minutes after 850 r of radiation. The results of this study indicate that the administration of 140 mgm./kgm. of 2-imino-thiazolidine-4-carboxylic acid 25 minutes after x-ray did not prevent the radiation-induced mortality from 850 r and appeared to enhance the lethal effects of x-irradiation as indicated by a reduction in the median survival time from nine days to five days.

TABLE 2

THE EFFECTS OF ORAL ADMINISTRATION OF MIXTURES OF CYSTEINE AND 2-MERCAPTOETHYLAMINE OR CYSTEINE AND AET ON THE CHANGES IN ENZYME ACTIVITIES OF THE SPLEENS, THYMUS GLANDS AND INTESTINES OF RATS THREE DAYS AFTER 400 r OF X-IRRADIATION

Combination	Dose in mgm./kgm.	Time of Administration Before X-ray (Minutes)	Spleen ATPase[a]		Thymus Glands ATPase[a]		Intestinal Cholinesterase[b]	
			400 r Activity	Effective % Dose Reduction	400 r Activity	Effective % Dose Reduction	400 r Activity	Effective % Dose Reduction
None	....	..	51.4±3.0	..	20.2±1.1	..	69± 9	..
Cysteine plus 2-mercaptoethylamine	1000 200	30	46.6±2.9	25	18.2±0.1	16	61± 3	0
Cysteine plus 2-mercaptoethylamine	1000 250	30	49.4±3.0	11	19.7±1.1	4	74±13	9
Cysteine plus AET	1000 300	30	49.1±1.1	12	16.4±0.5	25	69± 6	0
Cysteine plus AET	1000 350	30	48.2±1.6	17	21.3±0.7	0	77± 5	13
Cysteine plus AET	1000 450	30	47.2±0.9	19	18.7±0.5	11	91±15	28
Cysteine plus AET	1000 600	30	43.5±2.0	37	17.2±0.9	22	88±12	25
Cysteine plus AET	1000 700	30	43.9±1.1	36	20.1±1.6	0	77± 4	13

[a] Activity expressed as mgm. of P liberated from ATP/mgm. tissue/15 minutes.

[b] Activity expressed as µl. of CO_2 evolved/50 mgm. tissue/10 minutes.

TABLE 3

THE EFFECT OF 2-IMINO-THIAZOLIDINE-4-CARBOXYLIC ACID ON THE 30-DAY SURVIVAL OF RATS AFTER 850 r of TOTAL-BODY X-IRRADIATION

Dose in mgm./kgm.	Time of Administration with Respect to X-ray	Survivors/ Total Animals	% Survival	Median Survival Time (Days)
None	....	0/50	0	9
440[a]	10 min. before	3/5	60	23
440[a]	25 min. before	1/5	20	19
440[a]	25 min. after	0/5	0	5

[a]Expressed as mgm./kgm. equivalents of HCN.

Discussion

This investigation consisted of a continuation of experiments undertaken to obtain information concerning the radioprotective activity of 2-imino-thiazolidine-4-carboxylic acid injected intraperitoneally. Studies were also undertaken to determine the effect of the oral administration of various mixtures of cysteine and AET or cysteine and MEA on the radiation-induced changes in the adenosine triphosphatase activity of the spleens and thymus glands and in the cholinesterase activity of the small intestine of rats. The results of these experiments showed that 140 mgm./kgm. of 2-imino-thiazolidine-4-carboxylic acid provides substantial protection to the spleen and intestine and protected 60% of the rats against the lethal effects of 850 r of x-ray. Initial experiments indicated that the administration of this thiazolidine derivative in combination with MEA reduced the radioprotective activity of MEA in the tissues studied.

Studies on the influence of mixtures of cysteine and AET given orally on the radiation-induced changes in the enzyme activity of the hematopoietic tissues and intestine of rats showed that the mixture of 1,000 mgm./kgm. of cysteine and 600 mgm./kgm. of AET provided a substantial reduction in the biological effects of 400 r in the tissues studied. Other doses of AET ranging from 300 mgm./kgm. to 700 mgm./kgm. were administered as mixtures with 1,000 mgm./kgm. of cysteine but were less effective in preventing the changes in enzyme activity of one or more of the tissues studied. Results of initial experiments on the radioprotective activity of 200 mgm./kgm. or 250 mgm./kgm. of MEA given orally in combination with 1,000 mgm./kgm. of cysteine indicated that this combination is considerably more effective when given intraperitoneally. Additional experiments are currently in progress to obtain further information on the influence of oral administration of various combinations of radioprotective compounds.

Summary

1. A study was conducted to determine the influence of 2-imino-thiazolidine-4-carboxylic acid on the radiation-induced changes in the enzyme activity of the spleens, thymus glands and intestines of rats. It was found that administration of 140 mgm./kgm. of the thiazolidine (expressed as equivalents of HCN) ten minutes before x-irradiation provided 19% and 16% reductions in the biological effect of 400 r in the spleens and small intestines respectively. Administration of this compound 25 minutes before radiation provided substantial protection to the spleens but did not benefit the other tissues studied. Increasing the dose of the thiazolidine to 175 mgm./kgm. failed to enhance the protective activity in the hematopoietic tissues. The combination of 140 mgm./kgm. of 2-imino-thiazolidine-4-carboxylic acid and 200 mgm./kgm. of MEA did not reduce the biological effect of x-irradiation to the extent of MEA given singly.

2. Experiments were undertaken to obtain information concerning the radioprotective activity of mixtures of cysteine and MEA or cysteine and AET when administered orally. These studies indicated that mixtures of 1,000 mgm./kgm. of cysteine with 200 mgm./kgm. or 250 mgm./kgm. of MEA provided moderate reductions in the biological effect of radiation in the hematopoietic

tissues but did not benefit the intestines when given 30 minutes before x-ray. Doses of AET ranging from 300 mgm./kgm. to 700 mgm./kgm. were given in combination with 1,000 mgm./kgm. of cysteine. The mixture containing 1,000 mgm./kgm. of cysteine and 600 mgm./kgm. of AET was the most effective and provided reductions of 37%, 22% and 25% in the biological effects of x-irradiation in the spleens, thymus glands and intestines respectively.

3. Results of studies on the effects of 140 mgm./kgm. of 2-imino-thiazolidine-4-carboxylic acid on the 30-day survival of rats given 850 r of x-ray showed that the administration of this agent ten minutes before radiation permitted 60% survival. One of the five rats given the thiazolidine derivative 25 minutes before radiation survived the test period. Administration of the compound 25 minutes after 850 r of x-ray enhanced the lethal effects of radiation as indicated by a marked reduction of the median survival time.

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THE EFFECTS OF IONIZING RADIATIONS ON THE BIOCHEMISTRY OF MAMMALIAN TISSUES

III. Influence of X-irradiation on the Reductase Activity of the Livers of Partially Hepatectomized Rats

Kenneth P. DuBois and Bernard E. Hietbrink

This report concerns: Further measurements of the reductase activity of the livers of normal animals and a comparison of the rate of development of reductase activity in the livers of unirradiated and irradiated, hepatectomized rats.

Immediate or ultimate application of the results: The present study represents a continuation of studies on the effects of radiation on individual steps of the hydrogen transport system. A number of recent previous experiments in this laboratory have shown that the development of oxidative microsome enzymes that require reduced triphosphopyridine nucleotide are inhibited by x-irradiation. Since the reductase activity has the same coenzyme requirement as microsome oxidases, a study of the influence of radiation on reductase activity offered a means of ascertaining whether the radiation-induced defect in microsome enzyme activity occurred at a step preceding the formation of reduced triphosphopyridine nucleotide. Our previous studies have shown that x-irradiation does not inhibit the reductase activity of the livers of young rats. Previous studies in this laboratory demonstrated that the development of microsome oxidases in the regenerating livers of hepatectomized rats is inhibited by x-irradiation. In the present study the influence of x-irradiation on the development of reductase activity in the livers of hepatectomized rats was measured. It was anticipated that radiation would not affect the regeneration of this system in view of the absence of an effect by radiation on the reductase activity of the livers of young animals. The results of these experiments have provided further evidence for a radiation sensitive step in hydrogen transport system located between reduced triphosphopyridine nucleotide and oxidizable substrates in the microsome enzyme systems of rat liver. Thus, the findings have provided information which localizes to some extent the radiation-sensitive step in metabolic reactions catalyzed by microsome enzymes. The ultimate objective of these studies is to expand available information on the basic biochemical defects produced in animal tissues by ionizing radiations.

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A number of previous experiments in this laboratory have indicated that the development of oxidative reactions catalyzed by microsome enzymes is inhibited in the livers of young, male rats. Direct evidence for this effect was obtained (1) by measurements of the oxidative desulfuration of phosphorothioates. The development of these enzyme systems to the normal adult level normally occurs during the first six weeks after birth of male rats (2). Young rats exposed to 200 r or 400 r of x-irradiation at the age of 23 days failed to exhibit the increase in enzyme activity that normally occurs between 23 and 45

days of age (1). Testosterone stimulates the development of this system (3) in the livers of male rats but shielding the testes during irradiation did not prevent the radiation-induced inhibition of the development of the enzyme system.

The inhibitory effect of radiation on oxidative microsome enzymes stimulated our interest in further studies on microsome enzymes. The activity of these enzymes is dependent upon reduced pyridine nucleotides which are generated by dehydrogenase systems. If radiation inhibits a reaction that normally produces reduced pyridine nucleotides, all microsome enzymes dependent upon these coenzymes should be affected. Some direct measurements of this possibility were conducted in this laboratory by studying the glucose-6-phosphate dehydrogenase and 6-phosphogluconic dehydrogenase activity (4,5) of the livers of irradiated rats. Radiation did not inhibit any step in the hydrogen transport system from the substrate to reduced triphosphopyridine nucleotide sufficiently to account for the marked inhibition of the phosphorothioate oxidizing enzyme in the livers of young rats.

In further attempts to obtain information on the site of action of radiation on the development of liver microsome enzymes, we investigated the reductase activity of the livers of young, normal and irradiated rats. Prior to the radiation experiments a detailed study was conducted (6) on the procedure for quantitative assay of reductase activity. It was found that the method of Fouts and Brodie (7) does not satisfy the requirements for a valid enzyme assay when applied to rat liver. In this respect the most important factor was the conjugation of p-aminobenzoic acid formed by reduction of the p-nitrobenzoic acid which necessitates hydrolysis of the conjugate. After development of a suitable reductase method it was applied to the livers of normal adult male and female rats and male mice. The present report contains the results of additional measurements of the reductase activity of the livers of adult, female mice and adult, male guinea pigs.

Experiments conducted on young male rats sacrificed between the ages of 22 and 42 days demonstrated that the enzyme activity of the liver is less than half the adult level at 22 days of age and the activity reaches the adult level by 35 days of age. Rats exposed to 400 r of x-ray at 23 days of age exhibited no inhibition of the rate of development of reductase activity in the liver (6).

In a previous study (8) we found that sublethal doses of radiation inhibit the development of a microsome oxidase in the regenerating livers of male rats after partial hepatectomy. Radiation produced an effect in hepatectomized rats similar to that noted in young male rats. In view of the resistance of reductase to inhibition by radiation in young animals it was anticipated that the regeneration of reductase activity after hepatectomy would also be resistant to radiation. The preliminary experiments described in this report indicate that radiation does not affect the synthesis of this reduced triphosphopyridine nucleotide-linked enzyme.

Materials and Methods. Adult, Carworth Farms (CF_1) mice, young male guinea pigs and adult, male Sprague-Dawley rats were used for these experiments. The animals were kept in air-conditioned rooms and were fed Rockland Rat diet and water ad libitum. Partial hepatectomy was carried out under ether anesthesia.

X-irradiation was administered as single whole body exposure with a G.E. Maximar Therapy Unit. The radiation factors were 250 KVP, 15 ma., 0.25 mm. Cu and 1 mm. Al added filtration. The target-animal distance was 75 cm. and the dose rate was 35 r to 39 r per minute.

Measurement of reductase activity was made by the method developed recently in this laboratory (6). For the assays on mouse and guinea pig liver three levels of tissue were used (25 mgm., 50 mgm., and 100 mgm.). The assays on rat liver were performed using 25 mgm. and 50 mgm. of liver.

Results

Reductase activity of the livers of normal female mice and male guinea pigs. In previous studies (6) we found that there is no difference in the reductase activity of the livers of male and female rats and the enzyme activity of the livers of male mice is essentially the same as that of rats. In the present study assays were performed on the livers of female mice. To obtain information on possible species differences, the reductase activity of the livers of male guinea pigs was measured. The results of these measurements are summarized in Table 1 where the average and range of values for groups of four animals are presented. The values for the amount of free p-aminobenzoic acid in the medium and the total amount after acid hydrolysis for 0.5 hours are presented. The hydrolyzable conjugate is presumably the acetate.

TABLE 1

REDUCTASE ACTIVITY OF THE LIVERS OF FEMALE MICE AND MALE GUINEA PIGS

Species	Sex	Reductase Activity (µgm. of p-Aminobenzoic Acid/100 mgm. of Tissue/Hour)			
		Free		Total	
		Average	Range	Average	Range
Mice	Females	9.8	(9.3-10.2)	20.8	(18.9-22.7)
Guinea pigs	Males	4.7	(3.5- 5.4)	7.8	(7.1-8.6)

The results of these measurements showed that female mice exhibit somewhat lower reductase activity in the liver than males as evidenced by an average of 28.4 units of activity for males in our previous study (6). As was the case with males, a large part of the p-aminobenzoic acid was in the acetylated form. The average reductase activity of the livers of male guinea pigs was 7.8 units

which was about 3 to 4 times lower than the value for the livers of mice and rats. Furthermore, a comparison of the free and total p-aminobenzoic acid in the medium indicates that a smaller amount is acetylated in the case of guinea pig liver.

These measurements demonstrated that there is a considerable species difference in the reductase activity of the livers of guinea pigs and rats. The relative activity of the reductase activity of the livers of various species was not in the order which Fouts and Brodie (7) reported but these investigators did not consider the possibility that part of the formed p-aminobenzoic acid was acetylated in the reaction medium.

Influence of 200 r of x-irradiation on the development of reductase activity in regenerating rat liver. In a previous study (8) we found that the microsome enzyme system that catalyzes the oxidation of phosphorothioates returns to a normal level of activity in about 15 days after partial hepatectomy of rats. Sublethal doses of radiation given two days after partial hepatectomy markedly inhibited the rate of regeneration of the enzyme activity. To ascertain the effect of radiation on the reductase activity of regenerating liver a series of rats was partially hepatectomized. Some of the animals were exposed to 200 r of whole body x-irradiation at 18 hours after hepatectomy. Groups of three normal and irradiated rats were sacrificed at intervals after hepatectomy and the reductase activity of the liver was measured. The results of these measurements are summarized in Table 2.

TABLE 2

EFFECT OF X-IRRADIATION ON THE REDUCTASE ACTIVITY OF THE LIVERS OF PARTIALLY HEPATECTOMIZED RATS

Days After Hepatectomy	Dose of X-ray	Days After X-ray	Reductase Activity (μgm. p-Aminobenzoic Acid/100 mgm./Hour)			
			Free		Total	
			Average	Range	Average	Range
9	0	...	10.6	(10.2-10.8)	32.9	(30.7-34.3)
12	0	...	8.9	(8.8-9.0)	29.7	(28.7-31.7)
3	200 r	2-1/4	11.2	(11.1-11.4)	21.1	(18.9-22.8)
7	200 r	6	14.1	(11.5-14.8)	34.0	(30.4-37.6)
10	200 r	9	7.3	(6.6-7.6)	31.8	(28.9-35.0)

Although some additional assays remain to be performed on unirradiated animals sacrificed at shorter intervals after hepatectomy, the data in Table 2 suggest that the reductase activity returns to the normal level rapidly after partial hepatectomy. At intervals for which comparable data have been obtained on unirradiated and irradiated hepatectomized animals there was no difference in the reductase activity of the livers of the two groups. Thus it appears that radiation does not affect the regeneration of reductase activity in the livers of hepatectomized rats in contrast to the inhibitory effect on regeneration of microsome oxidase activity (8).

In the reductase assay developed for these studies nitrogen was used as the gas phase to prevent utilization of reduced triphosphopyridine nucleotide through oxidative pathways. Since at least one oxidative reaction that utilizes reduced triphosphopyridine nucleotide is inhibited by radiation (1), it seemed possible that the reduced triphosphopyridine nucleotide level might be maintained high enough even in the presence of oxygen for some reductase activity to occur. To test this possibility assays were performed on the same groups of animals used for the measurements shown in Table 2 and oxygen was used as the gas phase.

Comparison of the data in Tables 2 and 3 shows that the reductase activity was much lower under aerobic conditions than under anaerobic conditions. There was no increase in activity in the livers of irradiated animals. Thus, the livers of hepatectomized rats exposed to 200 r have sufficient capacity to oxidize triphosphopyridine nucleotide to prevent accumulation of reduced triphosphopyridine nucleotide.

TABLE 3

REDUCTASE ACTIVITY OF THE LIVERS OF PARTIALLY HEPATECTOMIZED, IRRADIATED AND UNIRRADIATED RATS MEASURED UNDER AEROBIC CONDITIONS

Days After Hepatectomy	Dose of X-ray	Days After X-ray	Reductase Activity (µgm. p-Aminobenzoic Acid/100 mgm. Tissue/Hour)			
			Free		Total	
			Average	Range	Average	Range
9	0	...	3.2	(2.4-3.8)	4.3	(4.2-4.4)
12	0	...	2.9	(2.2-3.6)	4.7	(4.1-5.2)
3	200	2-1/4	3.1	(2.6-4.0)	4.1	(3.7-4.5)
7	200	6	3.1	(2.3-4.1)	4.6	(3.3-5.9)
10	200	9	2.7	(2.6-2.8)	2.8	(1.9-3.0)

Discussion

The present investigation was conducted to obtain further information on the reductase system of rat liver and the influence of whole body irradiation on the reductase activity. Recent experiments in this laboratory (6) resulted in the development of a quantitative assay for reductase activity and application of the method to the tissues of normal animals. The results of assays on the livers of rats and mice demonstrated that there is no difference in the enzyme activity of the livers of male and female rats and male mice. In the present study it was found that the livers of female mice have slightly less activity than the livers of males and the livers of guinea pigs have much less reductase activity than mice or rats. Our studies demonstrated that the reductase activity is higher and species variations are different than was reported by Fouts and Brodie (7) but investigation of their procedure indicated that it does not meet the requirements of a valid enzyme assay.

Our previous experiments on reductase (6) have demonstrated that this enzyme is not affected by radiation in contrast to the marked inhibitory effect of radiation on the development of a microsome oxidase (1). Other experiments (4,5) showed that radiation does not appreciably affect any step in the glucose-6-phosphate dehydrogenase system between the substrate and reduced triphosphopyridine nucleotide. The results obtained to date, therefore, indicate that radiation inhibits a microsome enzyme reaction between an oxidizable substrate and reduced triphosphopyridine nucleotide. The exact nature of this oxidative enzymatic reaction has not been elucidated. Further work on the inhibitory effect of radiation on this system may aid in understanding the details of microsome oxidase reactions and may provide information on the exact mechanism by which radiation inhibits the reaction.

Summary

1. Measurements of the reductase activity of the livers of female mice indicated that the enzyme activity was slightly lower than the activity of the livers of male mice. The enzyme activity of the livers of normal male guinea pigs was 25% to 35% of the level of activity in the livers of rats and mice.

2. The reductase activity of the livers of partially hepatectomized male rats was measured on unirradiated rats and on comparable animals given 200 r of x-irradiation 18 hours after hepatectomy. No difference was observed between the enzyme activity of the irradiated and unirradiated animals. Conduction of the reductase assay under aerobic conditions also did not reveal any differences between irradiated and control animals thus indicating that utilization of reduced triphosphopyridine nucleotide through oxidative reactions was not sufficiently inhibited by this dose of radiation to cause accumulation of reduced triphosphopyridine nucleotide.

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PHARMACOLOGICAL AND TOXICOLOGICAL COMPOUNDS AS PROTECTIVE OR THERAPEUTIC AGENTS AGAINST RADIATION INJURY IN EXPERIMENTAL ANIMALS

I. The Influence of Various Chemical Compounds on Radiation Lethality in Mice

V. Plzak, M. Root and J. Doull

This report concerns: The survival time and mortality of male CF_1 mice treated with various chemical compounds immediately prior to the administration of a lethal dose of whole-body x-irradiation.

Immediate or ultimate application of the results: To find chemical compounds capable of reducing or preventing mortality in x-irradiated animals and to elucidate some of the structure-activity relationships within groups of related chemical protective agents. Although none of the currently available radioprotective agents provide a practical solution to the problem of preventing acute radiation injury because of their toxicity or relative ineffectiveness, the study of these compounds and related derivatives provides the most logical approach to finding compounds with an improved therapeutic index. A better understanding of the precise structural configuration(s) responsible for maximal radioprotective activity with minimal toxicity would also be of considerable value in furthering our knowledge of the basic mechanisms of radiation damage in biological systems.

* * * * * * * * *

During the past three months thirty-four additional chemical compounds were evaluated for protective activity against the lethal effects of whole body x-ray exposure in mice. In addition, two biological preparations, an extract of high molecular weight bacterial lipopolysaccharide and a specially processed type of chlorophyll, were administered under varying conditions before and after a lethal dose of x-irradiation to ascertain whether they would exhibit radioprotective effects. Previously (1), propylene glycol had been found to be protective when administered chronically and it was retested at this time to substantiate the results of the previous study.

Materials and Methods. Adult, male CF_1 Carworth Farms mice were employed for these studies. The compounds were dissolved either in water or in propylene glycol and were administered intraperitoneally with the concentration adjusted so that the animals received no more than 1% of their body weight with each injection. Preliminary toxicity studies were carried out with each compound to determine the maximum amount of each derivative which could be administered to the mice without causing mortality due to the chemical toxicity.

At least two dosage levels of each derivative were employed for the radiation studies and a minimum of ten mice were tested at each dosage level. The compounds were administered 15 minutes prior to the x-ray exposure which consisted of 700 r of whole-body x-irradiation given as a single exposure.

The radiation factors were 250 KVP, 15 ma., target-skin distance 75 cm., added filtration 0.25 mm. copper plus 1.0 mm. aluminum; and the dose rate was 40 r per minute as determined by means of a Victoreen Ionisation chamber in air. Control animals were given comparable amounts of the vehicle and irradiated simultaneously with the treated animals. The mortality and the weight loss in the control and treated mice were followed daily for 30 days after the x-ray exposure or until all of the animals were dead. The detailed description of the irradiation procedure, housing and handling has been included in previous reports (2).

The USAF code letter designation and the source of the compounds included in this study are listed in Table 1.

TABLE 1

SOURCE AND USAF CODE NUMBER OF COMPOUNDS INCLUDED IN THIS REPORT

USAF Designation	Source of Compound
AL	Alpinapharm Ltd., Zurich, Switzerland
CY	Dr. E. Groth, American Cyanamid Company, Bound Brook, New Jersey
EK	Eastman Kodak Company, Rochester, New York
UI	Dr. N. J. Leonard, University of Illinois, Urbana, Illinois
GE	Dr. M. Weiner, Geigy Chemical Corp., Ardsley, New York
VI	Dr. H. S. Sadow, U. S. Vitamin and Pharmaceutical Corp., N. Y.
MJ	Dr. E. W. Durachta, Mead Johnson Research Center, Evansville, Ind.
WL	Dr. B. S. Swartz, Warner-Lambert Research Inst., Morris Plains, N.J.
PD	Dr. R. D. Westland, Parke Davis and Company, Detroit, Michigan
UCTL	University of Chicago Toxicity Laboratory

Results

Preliminary toxicity studies. In order to determine the maximum safe dose for use in the radiation studies, it was necessary to obtain an approximate LD_{50} for the various compounds. Accordingly, small groups of mice were injected intraperitoneally with increasing dosage levels of each compound, and the resulting mortality was recorded for a period of one week. The results of these toxicity tests are shown in Table 2.

Evaluation of compounds for radioprotective activity. Since the x-ray dose of 700 r used for these studies produces 100% mortality within a period of two weeks, a compound is considered to exhibit significant radioprotective activity if it increases the ST_{50} by over five days or if it permits any of the treated animals to survive for 30 days after the x-ray exposure. The results of the radiation studies may be seen in Table 2. Included are the name, number and structural formulae of each of the compounds, the vehicle used for both the toxicity and radioprotective studies, the increase or decrease (in days) in the

TABLE 2

ACUTE INTRAPERITONEAL TOXICITY AND RADIOPROTECTIVE ACTIVITY OF VARIOUS CHEMICAL COMPOUNDS IN MALE CF_1 MICE

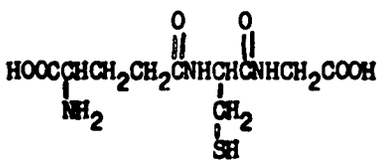
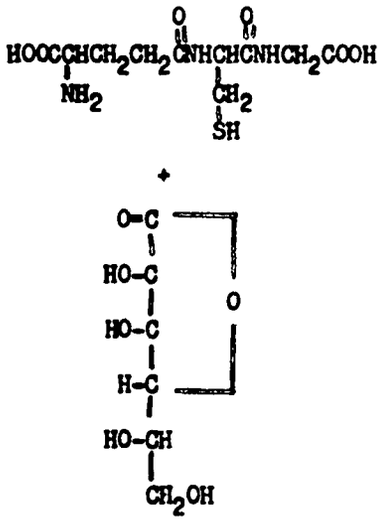
Name and Formula of Compound, USAF No. and Vehicle Used for Toxicity and Radiation Tests	Toxicity	Radiation Studies		
	Approx. LD_{50} in mgm./kgm.	Dose in mgm./kgm.	Change in ST_{50} in Days	Mortality at 30 Days After X-ray
Glutathione (glutamyl-cysteinyl-glycine) AL-1 (H_2O) 	>100	100 50	+ 2 0	9/10 10/10
Glutathione (63.57%)-ascorbate (36.43%) AL-2 (H_2O) 	>100	100 50	+ 2 + 1	9/10 9/10

TABLE 2--Continued

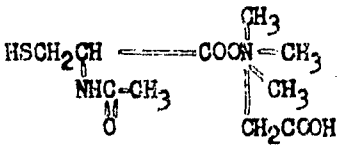
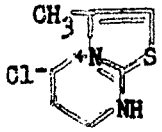
Name and Formula of Compound, USAF No. and Vehicle Used for Toxicity and Radiation Tests	Toxicity	Radiation Studies		
	Approx. LD_{50} in mgm./kgm.	Dose in mgm./kgm.	Change in ST_{50} in Days	Mortality at 30 Days After X-ray
Glutathione-ascorbate (one part glutathione to ten parts of vitamin C) AL-3 (H_2O)	>100	100 50	+ 2 - 1	10/10 10/10
Glutathione ascorbate with calcium phytate (calcium magnesium inositol hexaphosphate) AL-4 (H_2O)	>100	100 50	+ 2 - 2	10/10 10/10
(Carboxymethyl) trimethyl ammonium N-acetyl-L-cysteinate MJ-4 (5075-40) (H_2O) 	>1000	1000 500	0 0	10/10 10/10
6-7-Dihydro-3-methyl-5H-imidazo (2,1b)thiazolium chloride MJ-5 (6796-1) (H_2O) 	50-100	50 25	- 2 - 1	10/10 10/10

TABLE 2--Continued

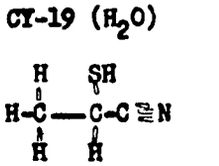
Name and Formula of Compound, USAF No. and Vehicle Used for Toxicity and Radiation Tests	Toxicity	Radiation Studies		
	Approx. LD_{50} in mgm./kgm.	Dose in mgm./kgm.	Change in ST_{50} in Days	Mortality at 30 Days After X-ray
Sodium beta-sulfopropionitrile CY-19 (H_2O)  (Na salt)	>1000	500 300	- 1 - 2	10/10 10/10
DBC Powder No. 2 VI-1 (H_2O) (A biguanide Phenformin derivative)	100	50 25	+ 3 + 3	9/10 10/10
DBB Powder No. 4 VI-2 (H_2O) (A biguanide Phenformin derivative)	50-100	50 25	+ 2 + 3	10/10 10/10
DBV Powder No. 6D VI-3 (H_2O) (A biguanide Phenformin derivative)	100-200	100 50	+ 5 + 3	9/10 10/10
DBTU Powder No. 8 VI-4 (H_2O) (A biguanide Phenformin derivative)	100-200	100 50	+ 2 + 1	10/10 10/10

TABLE 2--Continued

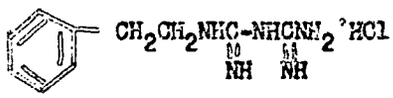
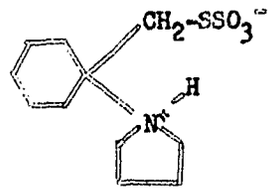
Name and Formula of Compound, USAF No. and Vehicle Used for Toxicity and Radiation Tests	Toxicity	Radiation Studies		
	Approx. LD_{50} in mgm./kgm.	Dose in mgm./kgm.	Change in ST_{50} in Days	Mortality at 30 Days After X-ray
DBCT Powder No. 1R VI-5 (H_2O) (A biguanide Phenformin derivative)	200-300	200 100 50	+11 + 2 + 2	3/5 10/10 9/10
Phenformin HCl No. 9113 (beta-phenethylbiguanide) VI-6 (H_2O) 	100-200	100 50 25	+ 2 - 1 + 1	4/4 10/10 10/10
1-(1-Pyrrolidinocyclo-hexyl)-methyl thiosulfuric acid (Zwitterion) UI-1 (I-179) (H_2O) 	100-200	100 50	+ 2 0	5/6 10/10
PD-30 (15620) (H_2O)	200-300	200 100	0 + 1	10/10 10/10
PD-33 (H_2O)	150-300	100 50	- 1 + 2	6/6 10/10

TABLE 2--Continued

Name and Formula of Compound, USAF No. and Vehicle Used for Toxicity and Radiation Tests	Toxicity	Radiation Studies		
	Approx. LD_{50} in mgm./kgm.	Dose in mgm./kgm.	Change in ST_{50} in Days	Mortality at 30 Days After X-ray
PD-35 (H_2O)	500	300 100 50	+ 2 + 4 + 2	4/5 10/10 10/10
PD-37 (H_2O)	500-1000	500 300	+ 4 + 2	6/10 10/10
PD-47 (H_2O)	25-50	25 10	+ 1 + 3	10/10 10/10
PD-48 (H_2O)	5-10	5 1	0 - 1	10/10 10/10
PD-75 (H_2O)	> 1000	1000 500	0 - 1	10/10 10/10
PD-76 (H_2O)	500-1000	500 300	- 1 - 1	10/10 10/10
PD-77 (PG)	500-1000	500 300	> 21 + 2	4/10 10/10
PD-78 (PG)	300-500	300 100	+ 2 - 3	9/10 10/10
PD-79 (PG)	500-1000	500 300	0 - 1	10/10 10/10
PD-80 (PG)	200	100 50	- 3 - 1	10/10 10/10

TABLE 2--Continued

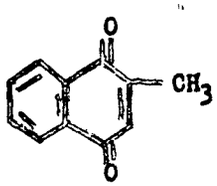
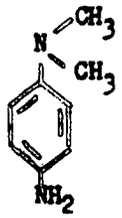
Name and Formula of Compound, USAF No. and Vehicle Used for Toxicity and Radiation Tests	Toxicity	Radiation Studies		
	Approx. LD_{50} in mgm./kgm.	Dose in mgm./kgm.	Change in ST_{50} in Days	Mortality at 30 Days After X-ray
PD-82 (PG)	300-500	300 100	+ 1 + 2	10/10 9/10
PD-97 (H_2O)	50-100	50 25	0 - 1	10/10 10/10
PD-98 (H_2O)	25-50	25 10	0 - 3	10/10 10/10
2-Methyl-1,4-naphthoquinone (menadione) EK-5185 (PG) (also B-140) 	50	50 25	- 5 - 2	10/10 10/10
N,N-Dimethyl-p-phenylene-diamine dihydrochloride EK-7423 (H_2O) 	25-50	25 10	+ 2 0	10/10 10/10

TABLE 2--Continued

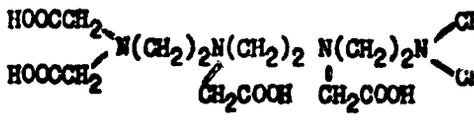
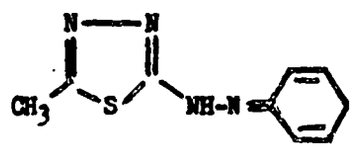
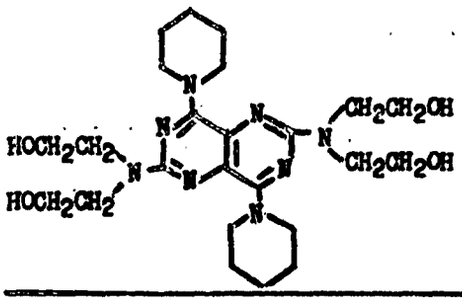
Name and Formula of Compound, USAF No. and Vehicle Used for Toxicity and Radiation Tests	Toxicity	Radiation Studies		
	Approx. LD_{50} in mgm./kgm.	Dose in mgm./kgm.	Change in ST_{50} in Days	Mortality at 30 Days After X-ray
GE-10 (37981) (H_2O) 		300 complexed with $CaCl_2$ (0.6-1.0)	- 3	10/10
		300 with $NaHCO_3$	0	10/10
	H_2O with $NaHCO_3$ >300	200 complexed with $CaCl_2$	- 1	10/10
	H_2O with $CaCl_2$ 300-500	200 with $NaHCO_3$	- 3	10/10
		200 with $NaHCO_3$ $CaCl_2$ pre	- 1	10/10
GE-11 (38306) (PO) 	50-100	50	- 2	10/10
		25	- 2	10/10
2,6-bis-(diethanolamino)-4,8-dipiperidino-pyrimido-(5,4-d) (Persantin) GE-12 (PO) 	100-200	100	- 1	10/10
		50	- 1	10/10

TABLE 2--Continued

Name and Formula of Compound, USAF No. and Vehicle Used for Toxicity and Radiation Tests	Treatment*	Radiation Studies		
		Dose in mgm./kgm.	Change in ST_{50} in Days	Mortality at 30 Days After X-ray
WL-1 (WXa 3148) An extract of high molecular weight bacterial lipopoly-saccharide	14 days pre 700 r	200	+ 1	10/10
	8 days pre 700 r	200	- 3	10/10
	3 days pre 700 r	200	- 4	10/10
	14 days pre 700 r	50	- 1	10/10
	8 days pre 700 r	50	- 1	10/10
	3 days pre 700 r	50	0	10/10

*Doses, concentrations and time of administration were suggested by Dr. Swartz.

ST_{50} of the treated mice in comparison with that of the simultaneously irradiated controls and the mortality at 30 days after the x-ray exposure.

Since glutathione has been shown to be an effective radioprotective agent in mice, it was of interest to evaluate preparations which were comprised of either glutathione alone and glutathione in various combinations with ascorbic acid and calcium-magnesium phytate. In Figure 1 it may be seen that when glutathione (AL-1) was administered at a dosage level of 100 mgm./kgm. prior to a lethal dose of x-irradiation, 10% of the mice survived for 30 days. Lowering the dose to 50 mgm./kgm. resulted in loss of the protective effect. When glutathione was given in combination with ascorbic acid (63.57%-36.43%) (AL-2), protection was also obtained. Both doses of AL-2 were beneficial with 10% of the mice surviving for 30 days after irradiation. The combination of glutathione and ascorbic acid in a 1:1 ratio (AL-3) showed no protection and the addition of CaMg inositol hexaphosphate to the combination (AL-4) likewise was ineffective.

Six biguanide derivatives, including β-phenethylbiguanide (Phenformin hydrochloride)(VI-6) were evaluated in the present studies. All of these compounds are oral hypoglycemic agents. Figure 2 shows the radioprotective effect obtained when VI-1 and VI-3 were administered prior to a lethal dose of whole body x-irradiation in mice. Ten per cent of the animals survived for 30 days when 50 mgm./kgm. of VI-1 and 100 mgm./kgm. of VI-3 respectively were used. A lower dose of 25 mgm./kgm. of VI-1 and 50 mgm./kgm. of VI-3 increased the ST_{50} but did not permit any of the animals to survive the 30-day post-irradiation period. VI-5 was administered at three dosage levels since the highest dose (200 mgm./kgm.) was quite toxic as evidenced by the death of five of the treated mice immediately following radiation. Of the five animals which were left, however, two survived for 30 days. No protection was seen when 100 mgm./kgm. of VI-5 was administered, but 50 mgm./kgm. of this compound did protect (10% 30-day survival). Figure 3 shows the results obtained with these agents.

Bunte salts have been found to exhibit beneficial effects against the lethality of whole body x-irradiation in experimental animals and compounds of this type are of interest in our program. In the present studies 1-(1-pyrrolidinocyclohexyl)-methyl thiosulfuric acid (UI-1), was administered at dosage levels of 100 mgm./kgm. and 50 mgm./kgm. respectively. Only the higher level was protective, but four of the ten mice given this dose succumbed immediately following the x-ray exposure. One of the four remaining mice lived for the 30-day period (Figure 3).

Since clearance has not yet been obtained for the next group of fifteen compounds, the names and structures of these agents are not listed. Of these fifteen compounds, five were radioprotective. Figures 4, 5, and 6 show the extent of this protection graphically.

Figure 7 shows the effect of administering an extract of high molecular weight bacterial lipopolysaccharides to mice at various times before an exposure to a lethal dose of whole-body x-irradiation. The doses, concentrations and time of administration for these studies were suggested by the supplier on the basis of previous work (3). WL-1 was given at doses of 200 mgm./kgm. and 50 mgm./kgm. respectively and at 14, 8, and 3 days prior to the x-ray exposure so that the resistance-increasing activity of the lipopolysaccharides toward levan-enhanced peritoneal injections in the mouse might be utilized to minimize the

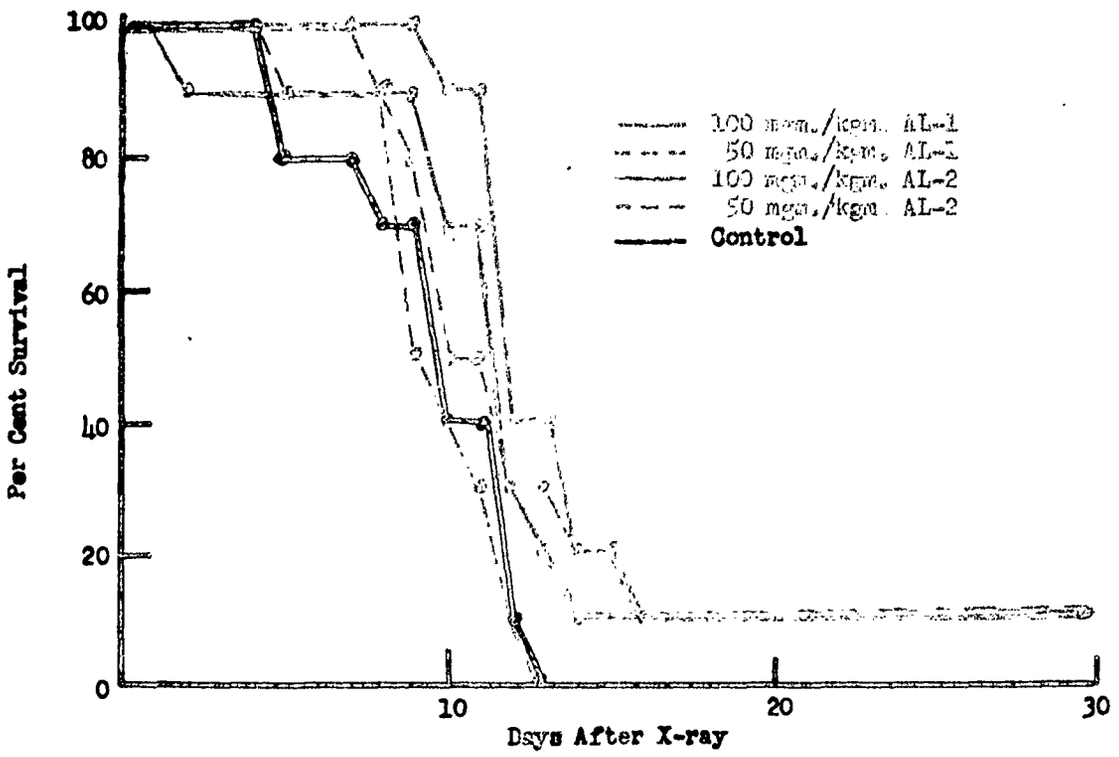


Figure 1. Effect of glutathione (AL-1) and glutathione-ascorbate (AL-2) on survival of mice irradiated with 700 r of whole-body x-irradiation.

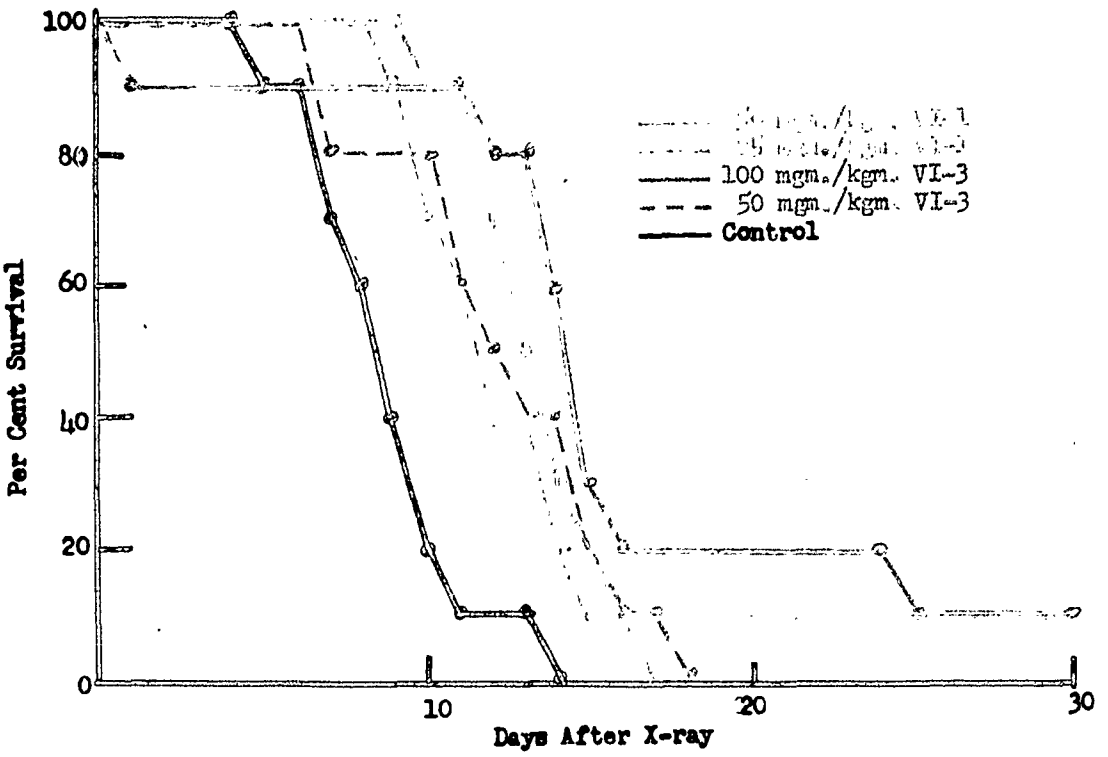


Figure 2. Effect of VI-1 and VI-3 on survival of mice irradiated with 700 r of whole-body x-irradiation.

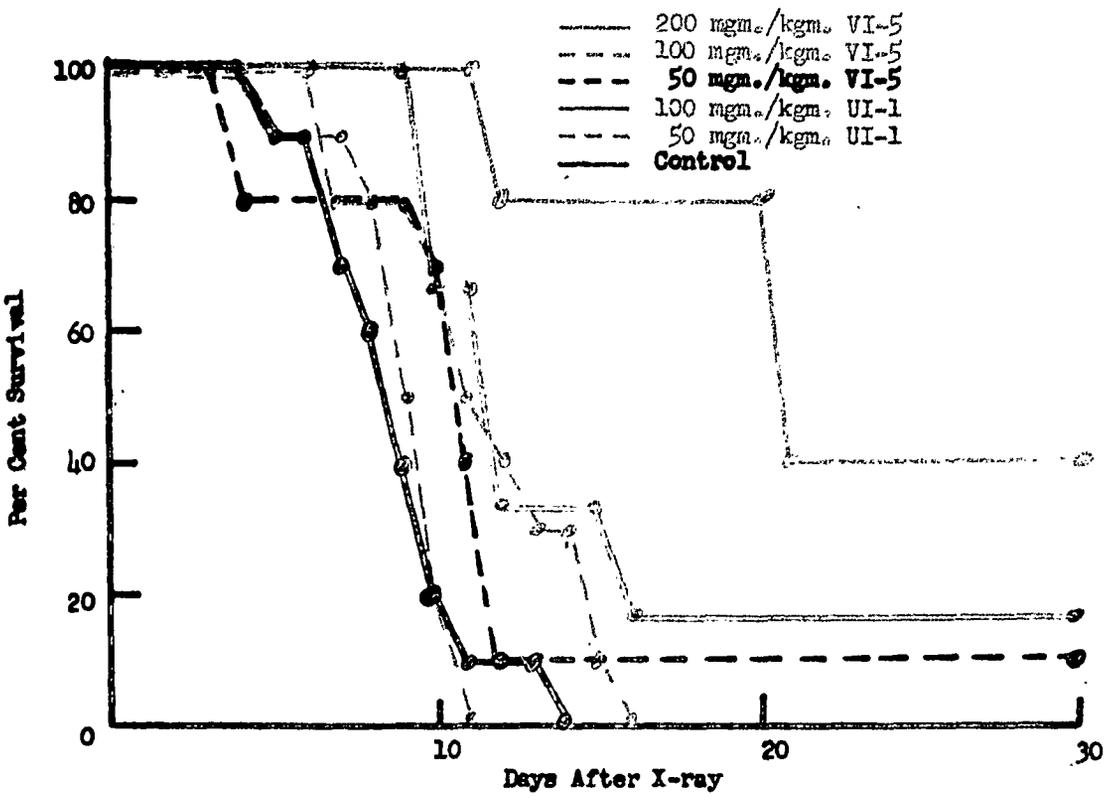


Figure 3. Effect of VI-5 and 1-(1-pyrrolidinocyclohexyl)-methyl thiosulfuric acid (UI-1) on survival of mice irradiated with 700 r of whole body x-irradiation.

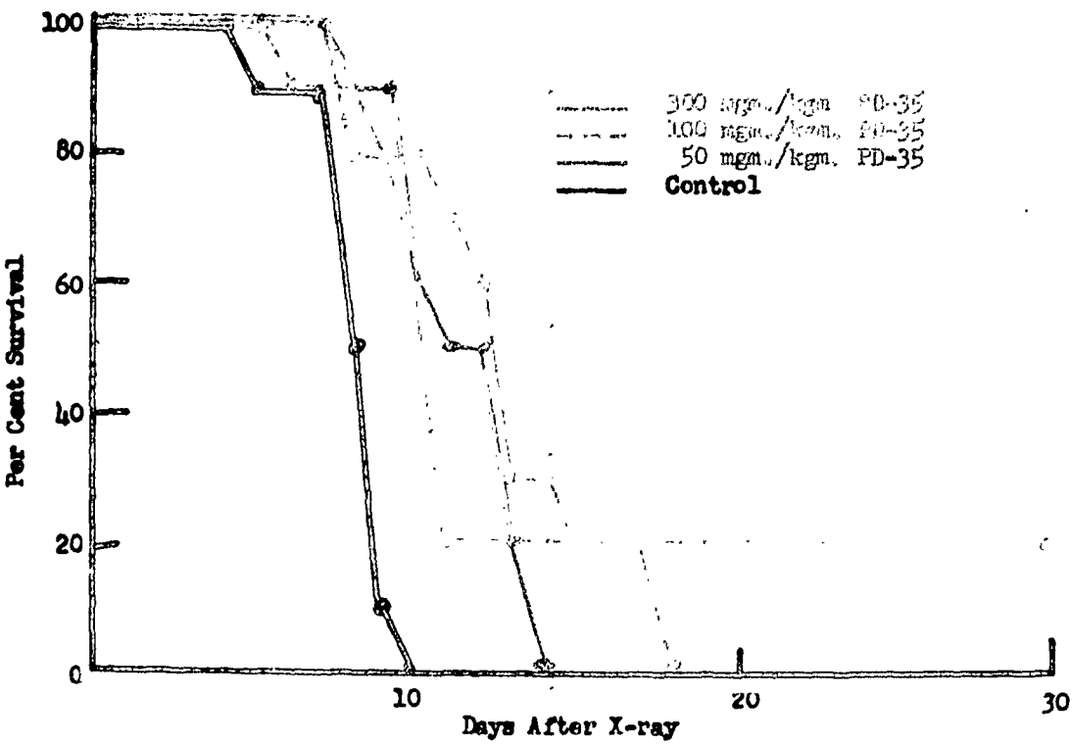


Figure 4. Effect of PD-35 on survival of mice irradiated with 700 r of whole body x-irradiation.

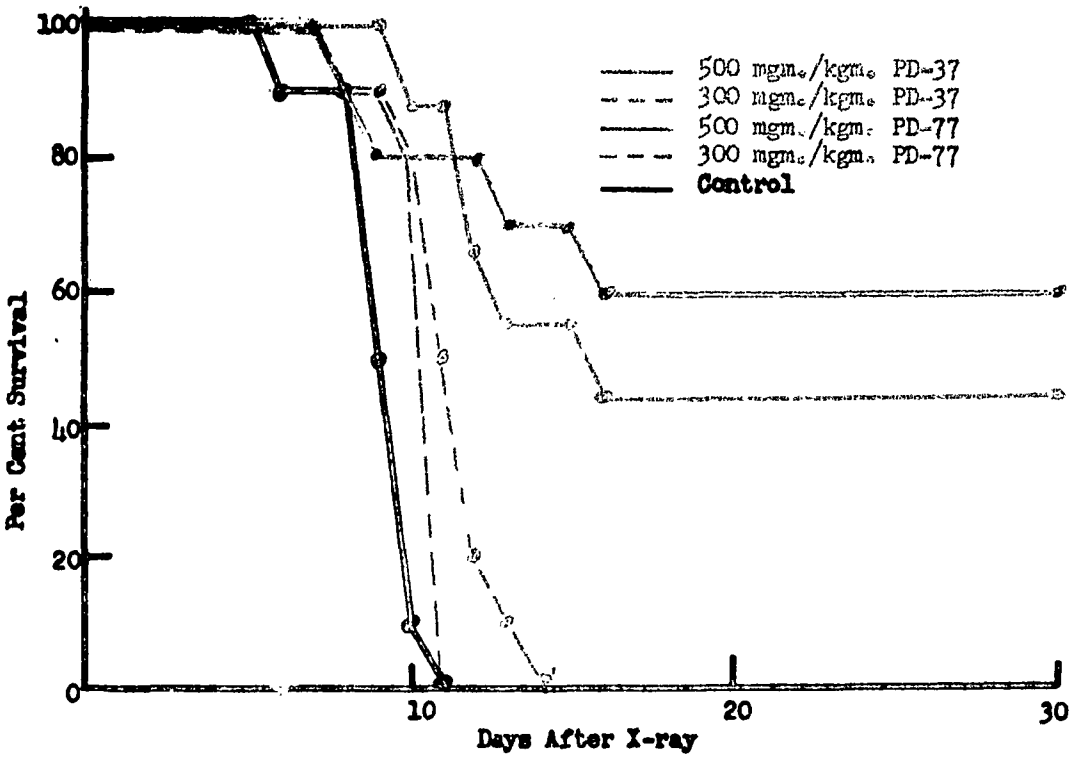


Figure 5. Effect of PD-37 and PD-77 on survival of mice irradiated with 700 r of whole-body x-irradiation.

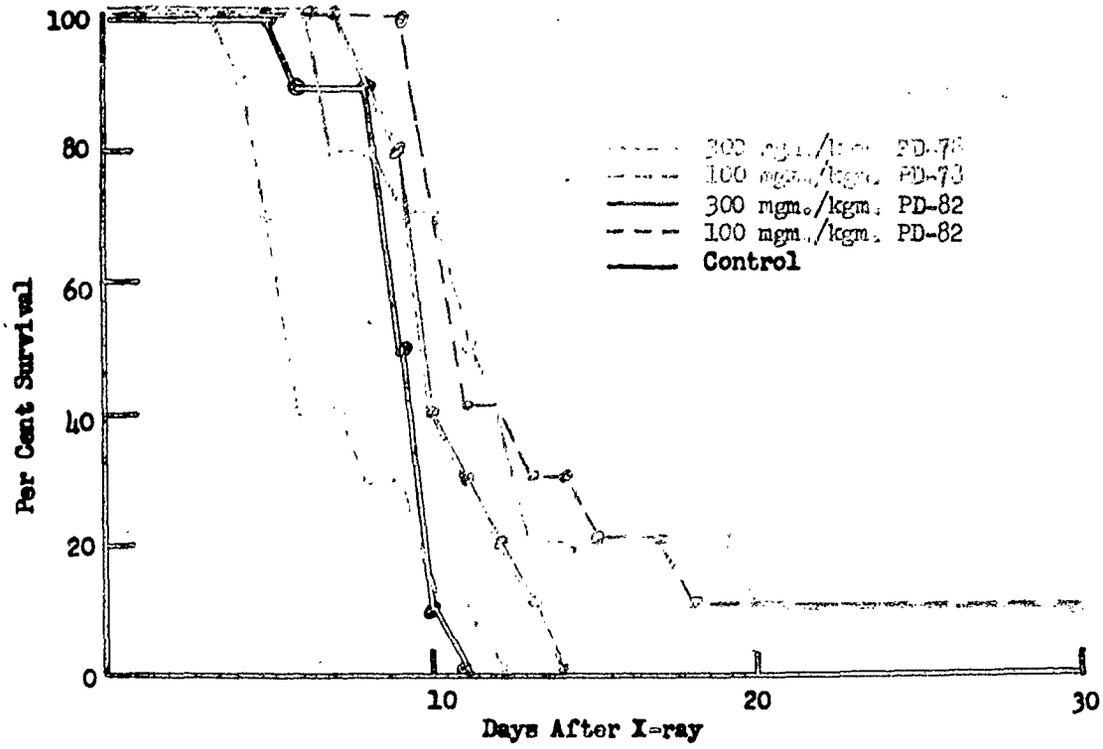


Figure 6. Effect of PD-78 and PD-82 on survival of mice irradiated with 700 r of whole-body x-irradiation.

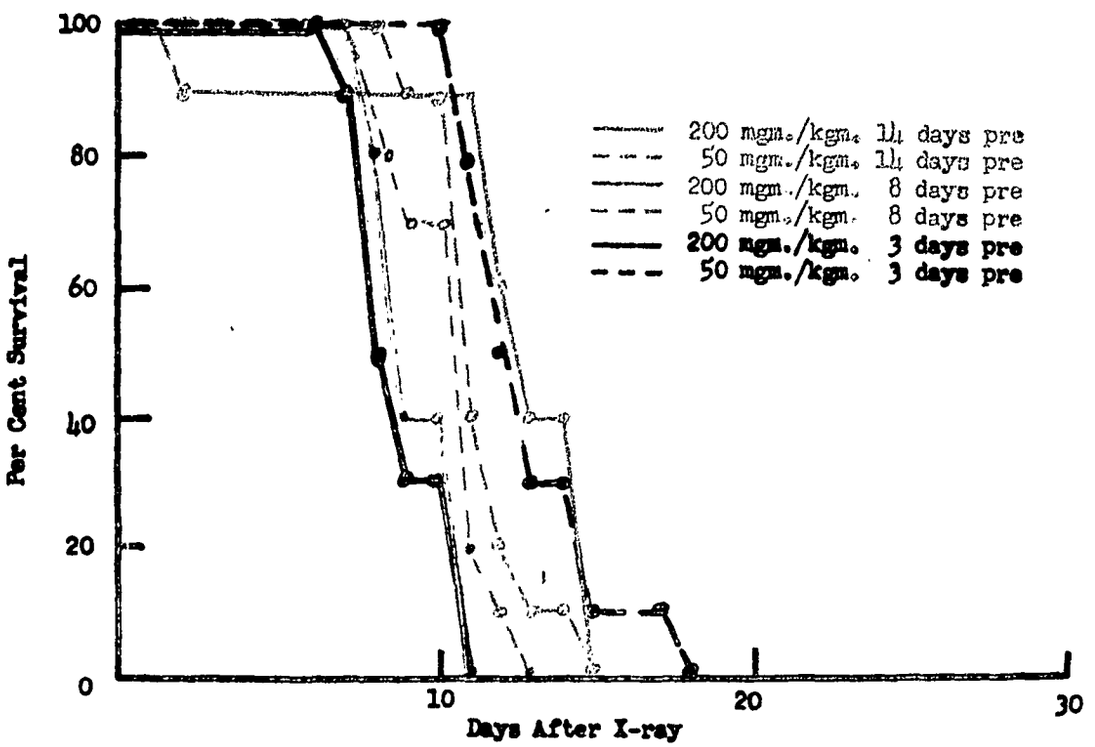


Figure 7. Effect of WL-1 on survival of mice irradiated with 700 r of whole-body x-irradiation

deleterious effects of radiation injury. Using these experimental conditions no radioprotective effect was evident; in fact, the ST_{50} of the treated mice was shortened when compared with that of the control animals, and none of the mice survived for the 30-day post-radiation period.

Chlorophyll (UCTL-21) extracted by a process which maintains the natural plant pigment entities in their undenatured form was tested for possible beneficial effects against the deleterious action of whole-body x-irradiation. For these studies various doses of UCTL-21 were administered intraperitoneally before and after a lethal exposure of 700 r. Figure 8 shows the results obtained when 10 mgm./kgm., 20 mgm./kgm. and 100 mgm./kgm. respectively were given to mice which were subsequently x-irradiated ten minutes later. It may be seen that in one instance 10 mgm./kgm. of UCTL-21 protected 30% of the mice for 30 days after the radiation exposure and 100 mgm./kgm. of UCTL-21 protected 20% of the mice for a similar period. Figure 9 shows the results of administering UCTL-21 after radiation, both as a single dose and chronically. An immediate post-irradiation injection of 10 mgm./kgm. allowed 10% of the mice to live for 30 days. When 30 minutes elapsed between the x-ray exposure and the administration of the chlorophyll, no beneficial effect was apparent. Chronic injections of 10 mgm./kgm. for five days following the x-ray resulted in a 30-day survival of 10% of the mice. Increasing the chronic dose to 100 mgm./kgm. not only decreased the protective effect but also shortened the lives of the treated animals.

In a previous study (1) the control group for a series of compounds given chronically prior to radiation exposure consisted of ten male mice given propylene glycol daily for four days prior to the x-ray exposure. Four of these propylene glycol treated mice survived for 30 days after the lethal x-ray exposure and since propylene glycol has only a very slight protective effect when given acutely prior to x-ray exposure (10% or less for 30-day survival), it was of interest to confirm the apparent radioprotective effect of chronic administration of this agent. For these studies ten male mice were given 0.5% of their body weight of propylene glycol daily for four days and four hours after the last injection they were exposed to 700 r of x-irradiation. The results of these studies are shown in Figure 10 where it can be seen that the protective effect of this treatment has been confirmed. It is planned to extend these observations and to investigate the effect of chronic administration of other glycols on radiation lethality in mice.

2-Mercaptoethylamine (MEA) is used extensively in this and other laboratories as a standard radioprotective agent. Mice treated with this agent are included in almost every series of radiation protection studies to detect variations in the quality of the animals, their post-irradiation care and in the various radiation factors. Over the years the MEA used for these studies has been obtained from a variety of sources and it was of interest, therefore, to compare samples from several of these sources for protective activity. MEA from three sources was used for these studies in which groups of mice were given a relatively low dose of MEA (225 mgm./kgm. intraperitoneally) prior to the administration of 700 r of whole-body x-irradiation. The low dose of MEA was used for these studies in the hope that any differences in protective activity might be more evident. The first sample of MEA was obtained from the General Biochemical Company and is a part of the lot which is currently in use for most of the programs underway in this laboratory. The second sample is one of several obtained from the California Biochemical Corporation and was

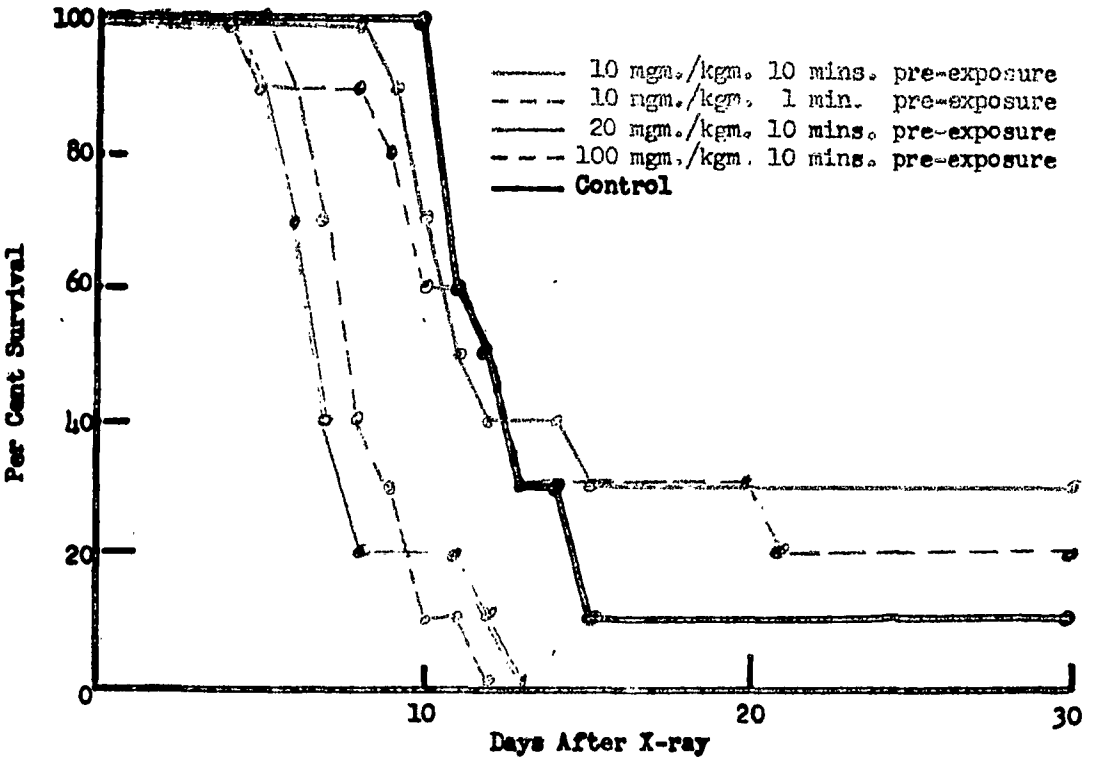


Figure 8. Effect of chlorophyll (UCTL-21) on survival of mice subsequently irradiated with 700 r of whole-body x-irradiation.

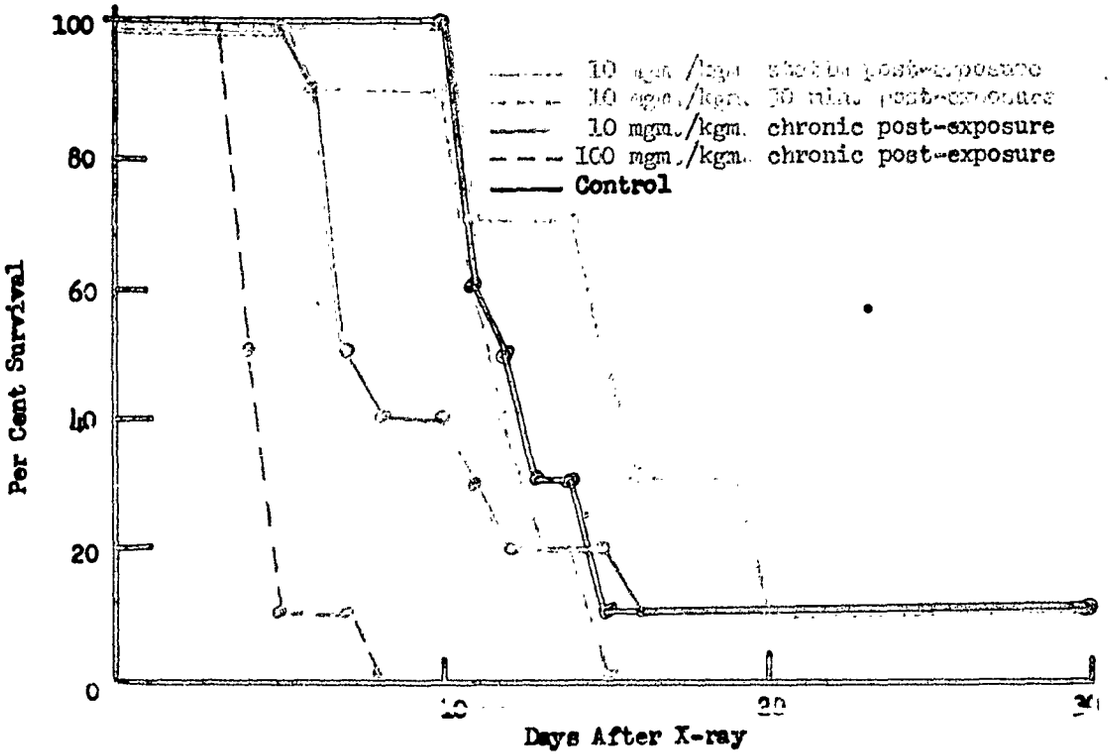


Figure 9. Effect of chlorophyll (UCTL-21) on survival of mice previously irradiated with 700 r of whole-body x-irradiation

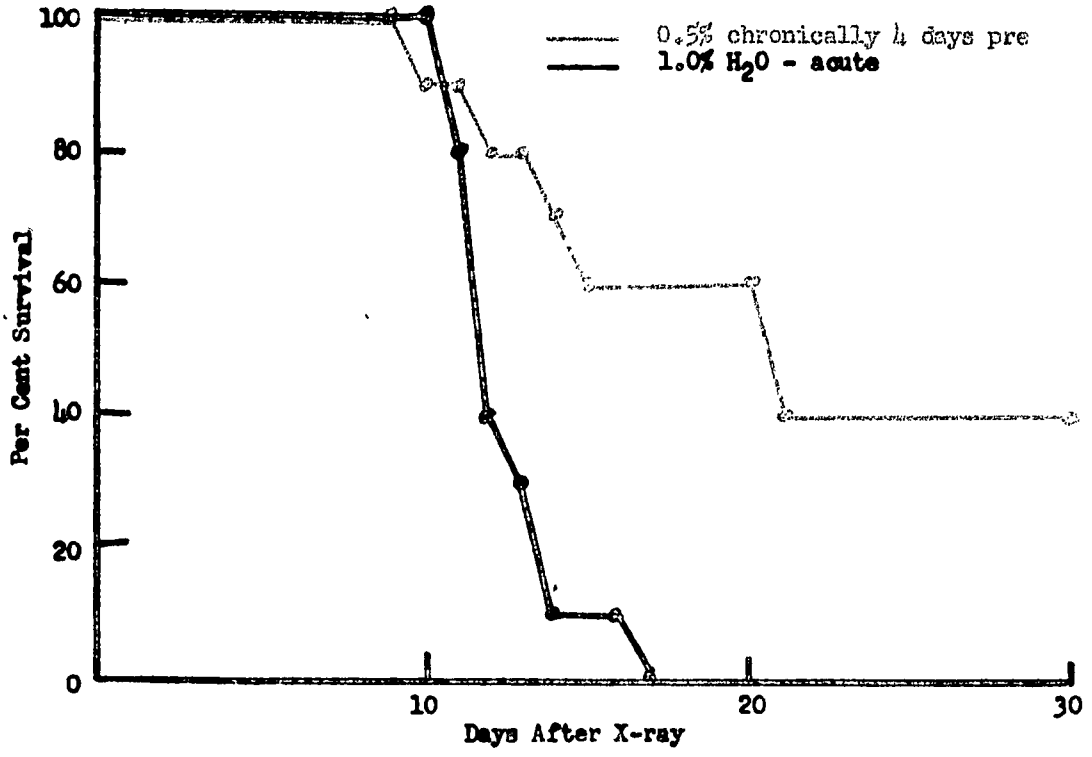


Figure 10. Effect of propylene glycol on survival of mice irradiated with 700 r of whole-body x-irradiation.

selected because it appeared to be slightly less soluble than most of the other samples of this agent. The third sample was a technical preparation of MEA obtained from Eastman Kodak Company. The results of these studies are shown in Figure 11 where it can be seen that 60% of the mice treated with the General Biochemical Company sample of MEA survived for 30 days after the radiation exposure, whereas only 10% of the animals treated with the sample obtained from California Biochemical Corporation survived for a similar period. There were no survivors in the group of mice which were treated with the same dose of MEA obtained from Eastman Kodak. Since animals treated with each of the three samples of MEA were irradiated simultaneously, it is unlikely that these differences can be attributed to variation in the radiation dose. It is also unlikely that the differences are due to variation in the animals since the mice were all selected from a single shipment and were housed together following the radiation exposure. We have previously obtained protective effects with the samples of MEA obtained from California Biochemical Corporation and Eastman Kodak when they were administered at higher dosage levels so that the samples are not simply mislabelled. No attempt was made to purify any of the three samples used for these tests and the results probably reflect the differences in purity between the samples.

Summary

The present report contains the results of toxicity and radioprotective evaluation studies on thirty-four additional chemical compounds in male CF_1 mice. Eleven of these compounds were found to exhibit significant radioprotective activity in that they reduced the 30-day mortality of the x-rayed mice. The most marked effect was obtained with PD-77 which allowed 60% of the mice to survive for 30 days after an otherwise lethal x-ray exposure. Two compounds protected 40% of the mice in a similar manner. They were VI-5, a biguanide derivative of Phenformin, and PD-37. PD-35 protected 20% of the x-rayed animals against the 30-day mortality and a bunte salt, 1-(1-pyrrolidinocyclohexyl)-methyl thiosulfuric acid (UI-1), protected 17%. The rest of the compounds found to be effective in these tests produced a 30-day survival of 10% of the mice given the whole-body x-ray exposure of 700 r. These compounds were: glutathione preparations, AL-1 and Al-2; Phenformin derivatives, VI-1 and VI-3; and PD-78 and PD-82.

An extract of high molecular weight bacterial lipopolysaccharides was administered to mice at various times prior to a lethal dose of x-ray using two different dosage levels. No protection was seen with any of the treatments used. An extract of chlorophyll was administered acutely before and after and chronically after an otherwise lethal dose of whole-body x-irradiation. Premedication with this material was effective in protecting 30% and 20% of the mice for 30 days when administered at dosage levels of 10 mgm./kgm. and 100 mgm./kgm. respectively. The post-treatment was effective when 10 mgm./kgm. was given either immediately following the x-ray exposure or when it was given chronically.

Propylene glycol was given chronically for four days prior to 700 r of whole body x-irradiation and found to be protective to 40% of the mice so pretreated.

Mercaptoethylamine hydrochloride, obtained from three different sources, was compared for radioprotective activity. Sixty per cent of the animals given

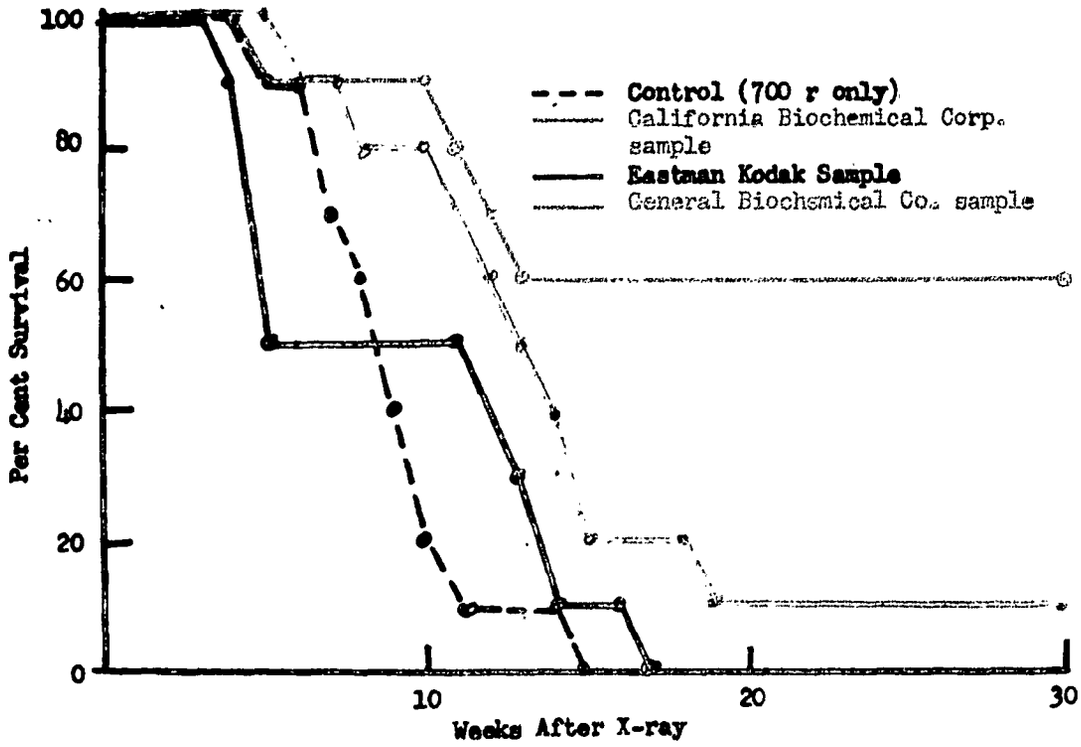


Figure 11. Comparison of the relative radioprotective activity of three samples of 2-mercaptoethylamine (MEA) against radiation lethality in CF_1 male mice.

the compound obtained from General Biochemical were protected against the lethal x-ray exposure while the compound obtained from California Biochemical protected only 10% of the mice and the Eastman Kodak product exhibited no protective activity.

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PHARMACOLOGICAL AND TOXICOLOGICAL COMPOUNDS AS PROTECTIVE OR THERAPEUTIC AGENTS AGAINST RADIATION INJURY IN EXPERIMENTAL ANIMALS

II. The Effects of Pre-irradiation and Post-irradiation Administration of Cyanide and Other Compounds on Radiation Lethality in Mice

J. Dilley and J. Doull

This report concerns: The survival time and mortality in male and female CF_1 mice treated with varying doses of cyanide prior to whole-body x-ray exposure and the therapeutic effectiveness of cyanide, 2-imino-thiazolidine-4-carboxylic acid, l-cystine, l-cysteine, potassium chloride and 2,2'-dithiobis (ethylamine) given 30 minutes after lethal whole-body x-ray exposure in mice.

Immediate or ultimate application of the results: To obtain information concerning the mechanisms(s) responsible for the radioprotective effects of cyanide and related compounds and, as a result, to obtain a better understanding of the biological effects of ionizing radiations. These studies constitute a part of a program designed to obtain information concerning both the toxic and protective effects of the currently available radioprotective agents. This type of information is essential for the evaluation of the potential usefulness of these agents and for the development of new agents or combinations of agents.

* * * * * * * * *

In previous studies (1,2) we have demonstrated that cyanide will protect mice against lethal doses of whole-body x-irradiation when given at dosage levels approaching the maximum tolerated dose immediately prior to the radiation exposure. In these studies it was also observed that a natural metabolite of cyanide (2-imino-thiazolidine-4-carboxylic acid) appeared to exhibit therapeutic effects against radiation lethality when it was given 30 minutes after radiation exposure. These observations have been extended and a variety of other possible metabolites and unreacted products have been tested for therapeutic activity against radiation lethality in mice and the results are presented in this report.

Materials and Methods. Adult, male Carworth Farms CF_1 mice weighing between 20 and 40 grams and adult, female Carworth Farms CF_1 mice weighing between 20 and 25 grams were used for these studies. The control and experimental animals were selected from single shipments, housed in groups of not more than ten animals per cage in an air-conditioned room (78° to 85° F.) and fed and watered ad libitum. Aqueous solutions of the compounds were freshly prepared just prior to use and were injected intraperitoneally in a volume which did not exceed 1% of the body weight.

The x-ray exposures were given by means of a Keleket X-ray Therapy unit operated at 250 KVP and 15 ma. with 1.0 mm. of aluminum, 0.25 mm. of copper and 1.0 cm. of lucite added filtration. The dose rate was determined prior to each exposure by means of a 250 r Victoreen Ionization Thimble in air. All of these studies were carried out at a dose rate of about 235 r per minute to insure that the irradiation period would be as short as possible. Both the control and the treated animals were irradiated simultaneously and the weight and mortality within each group was followed for 30 days or until the death of all of the mice in each group had occurred.

Results

Determination of the optimal dosage level of cyanide for maximal radioprotective effects in male and female mice. In previous studies (2) it was observed that an increased radioprotective effect (greater number of 30-day survivors) was obtained when the dosage level of cyanide (as HCN) was increased from 2.0 to 2.25 mgm./kgm. It was of interest, therefore, to determine whether the protective effect of this agent could be further increased if the dosage level was increased to near the LD_{50} level for cyanide. For these experiments groups of ten animals were injected with 2.25, 2.50 or 2.75 mgm./kgm. of cyanide (as HCN) four minutes prior to the administration of various doses of whole-body x-irradiation. The control animals for these studies were given comparable amounts of the vehicle (water). Potassium cyanide (Mallinkrodt, 96%) was used for these studies and the agent was given intraperitoneally in a volume equal to 1% or less of the body weight. The results of these studies are shown in Figures 1 through 8. The effect of the various doses of cyanide on the survival of x-irradiated female mice are summarized in Figures 1 through 4 and the results of similar studies in male mice are shown in Figures 5 through 8. It can be seen in Figures 2, 3, and 4 that the greatest number of animals surviving the lethal x-ray exposures were seen following the administration of 2.25 mgm./kgm. of cyanide. At this dosage level 75% of the animals exposed to 900 r survived for 30 days after the x-ray exposure whereas only 25% of the mice given 2.5 mgm./kgm. of cyanide survived for a similar period. The female mice treated with 2.75 mgm./kgm. of cyanide prior to the 900 r x-ray exposure exhibited a 30-day survival of only 12.5%. In contrast to these results, the maximum radioprotective effect in male CF_1 mice exposed to lethal doses of whole-body x-irradiation occurred when the cyanide was given at a dosage level of 2.5 mgm./kgm. In the mice given 950 r of whole-body x-irradiation, there was a 30-day survival of 40% in the group protected with 2.50 mgm./kgm. of cyanide whereas the administration of 2.25 or 2.75 mgm./kgm. of cyanide produced 30-day survivals of only 20% and 0% respectively. Although the differences in optimal dosage level for the male and female animals are not marked, it appears from these studies that the optimal radioprotective dosage level of cyanide is somewhat greater in male than in female mice. We have previously shown that the approximate LD_{50} for cyanide in male and female mice is about 3.25 mgm./kgm. (as HCN) and that there is little difference in the toxicity of this agent to male and female CF_1 mice. It is evident from the present studies that the radioprotective effect of cyanide is not directly proportional to the administered dose of cyanide when doses near the LD_{50} are employed. None of the animals given the cyanide in the present studies died as a result of cyanide toxicity.

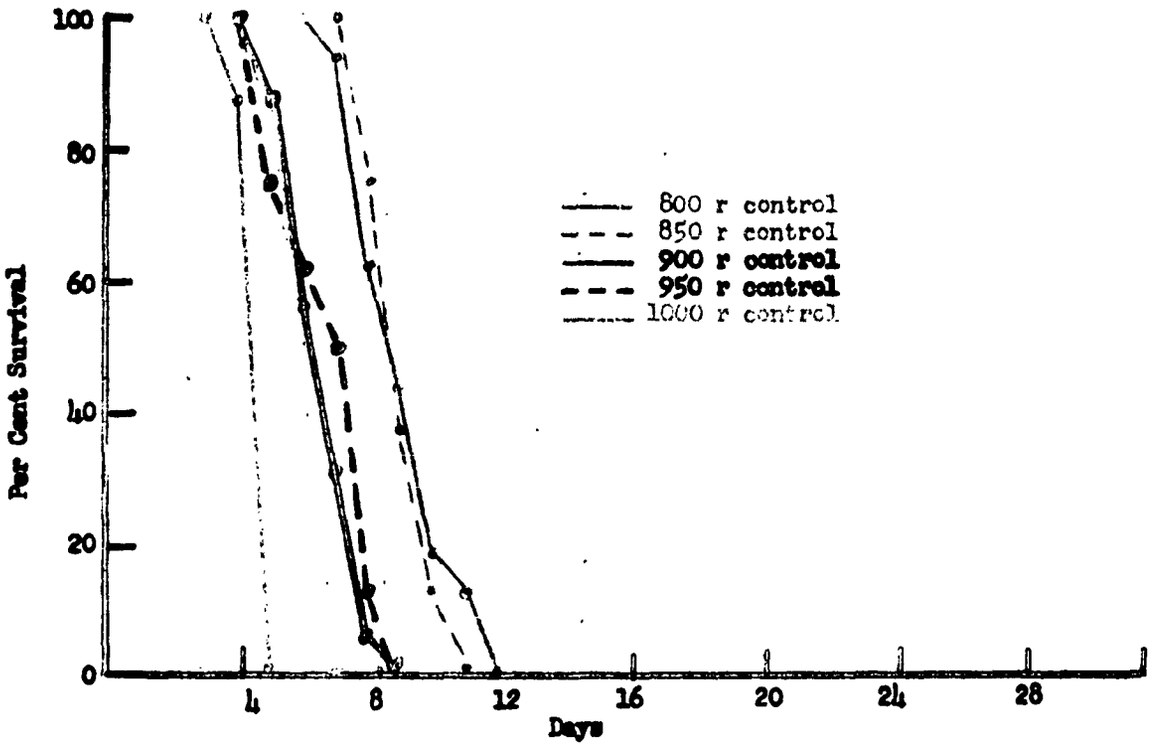

Figure 1. Survival time and mortality in CF_1 female mice exposed to various doses of whole-body x-irradiation.

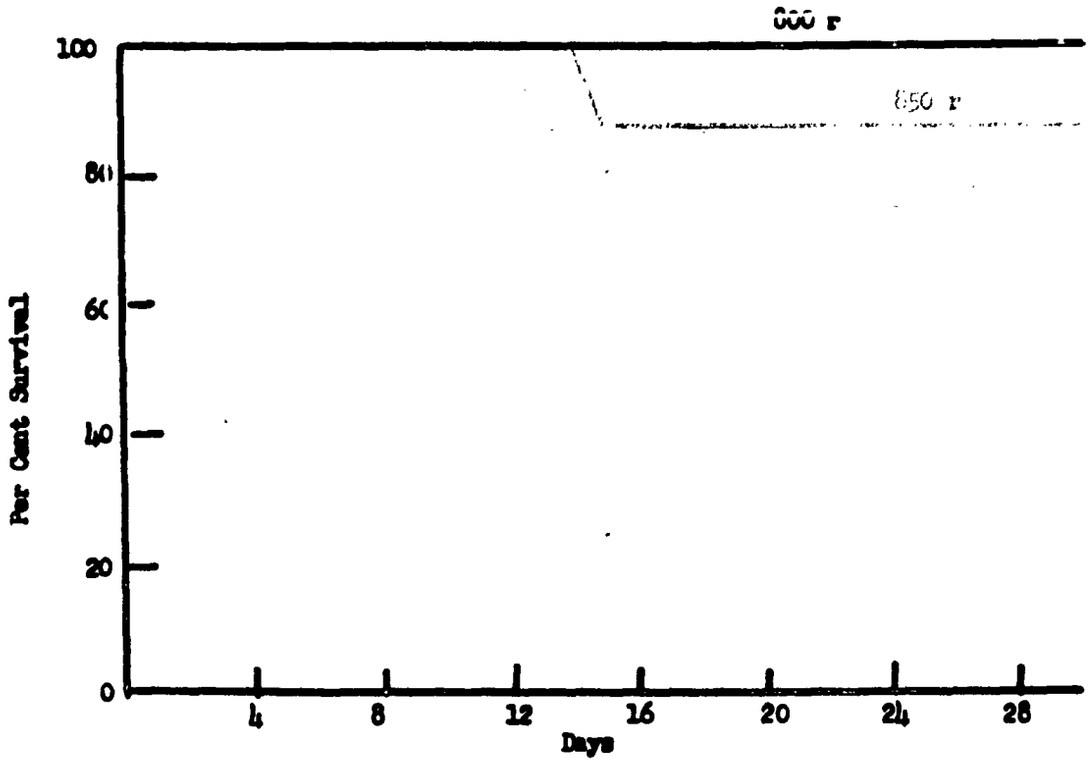

Figure 2. Survival time and mortality in CF_1 female mice given 2.25 mgm./kgm. of cyanide prior to the administration of various doses of whole-body x-irradiation.

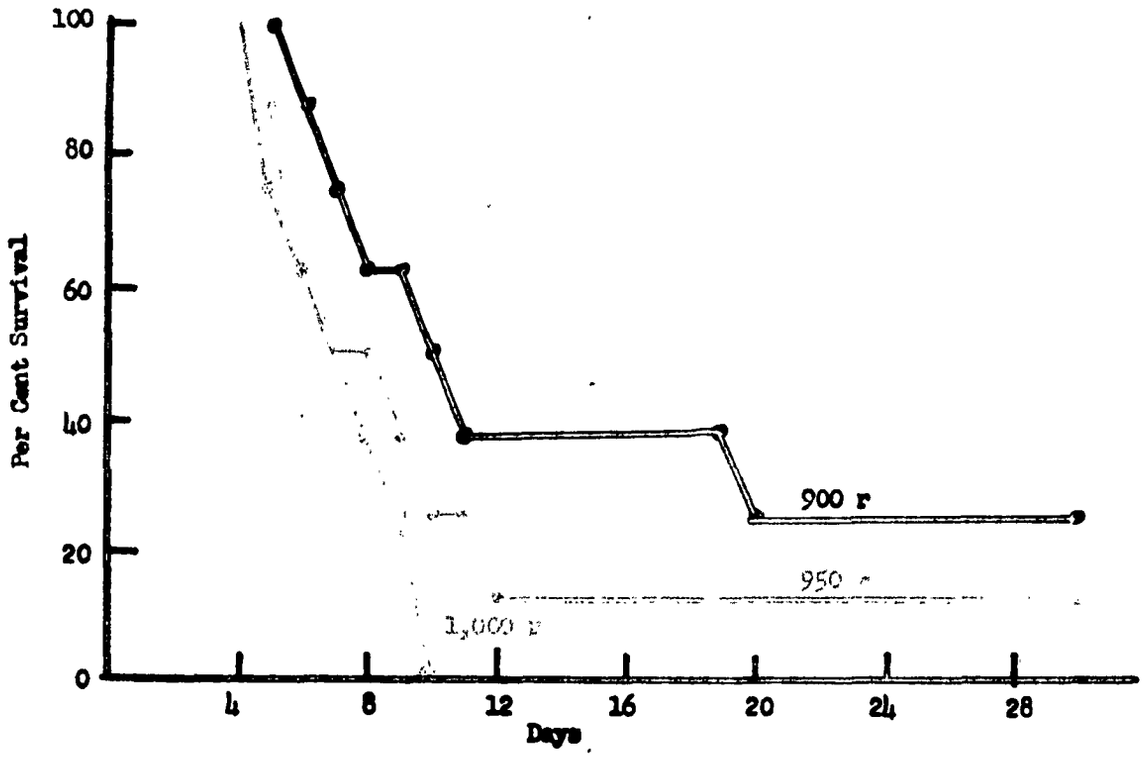

Figure 3. Survival time and mortality in CF_1 female mice given 2.50 mgm./kgm. cyanide prior to the administration of various doses of whole-body x-irradiation.

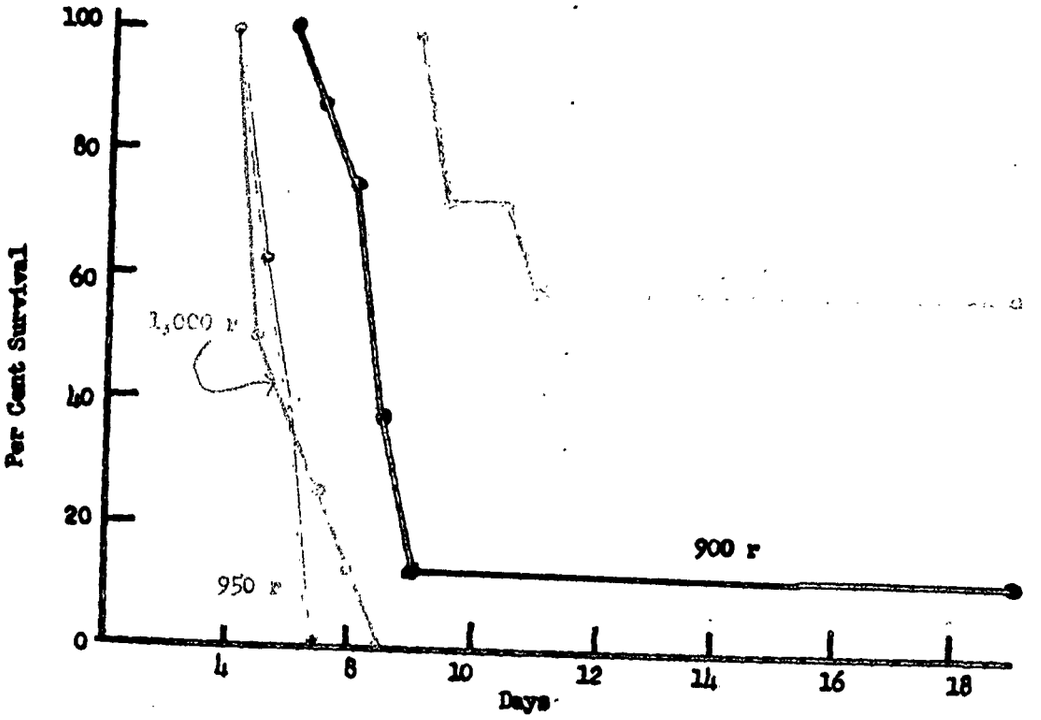

Figure 4. Survival time and mortality in CF_1 female mice given 2.75 mgm./kgm. of cyanide prior to the administration of various doses of whole-body x-irradiation.

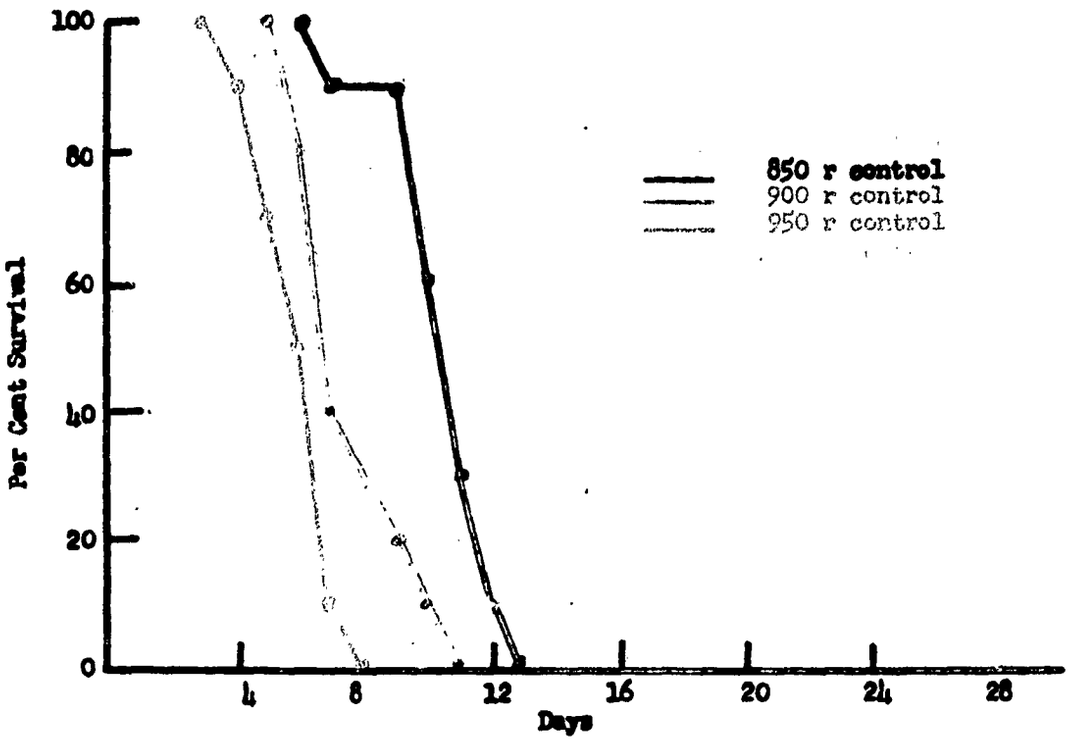

Figure 5. Survival time and mortality in CF_1 male mice given various doses of whole-body x-irradiation.

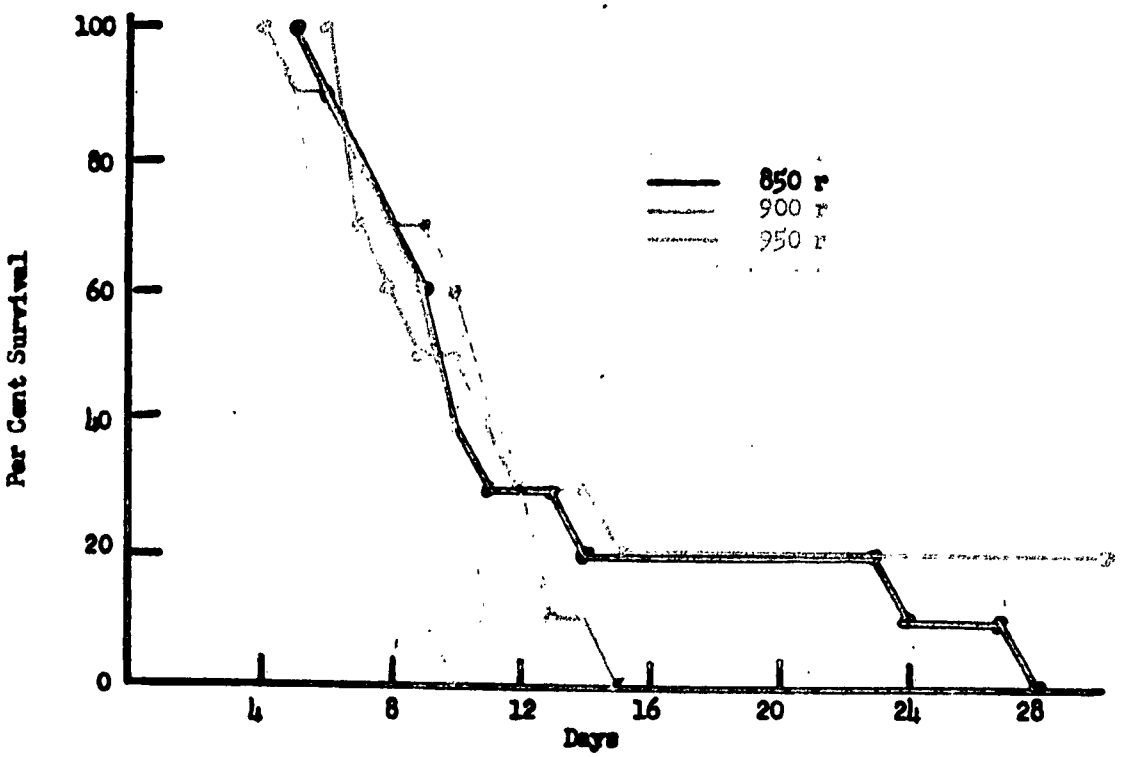

Figure 6. Survival time and mortality in CF_1 male mice given 2.25 mgm./kgm. of cyanide prior to the administration of various doses of whole-body x-irradiation.

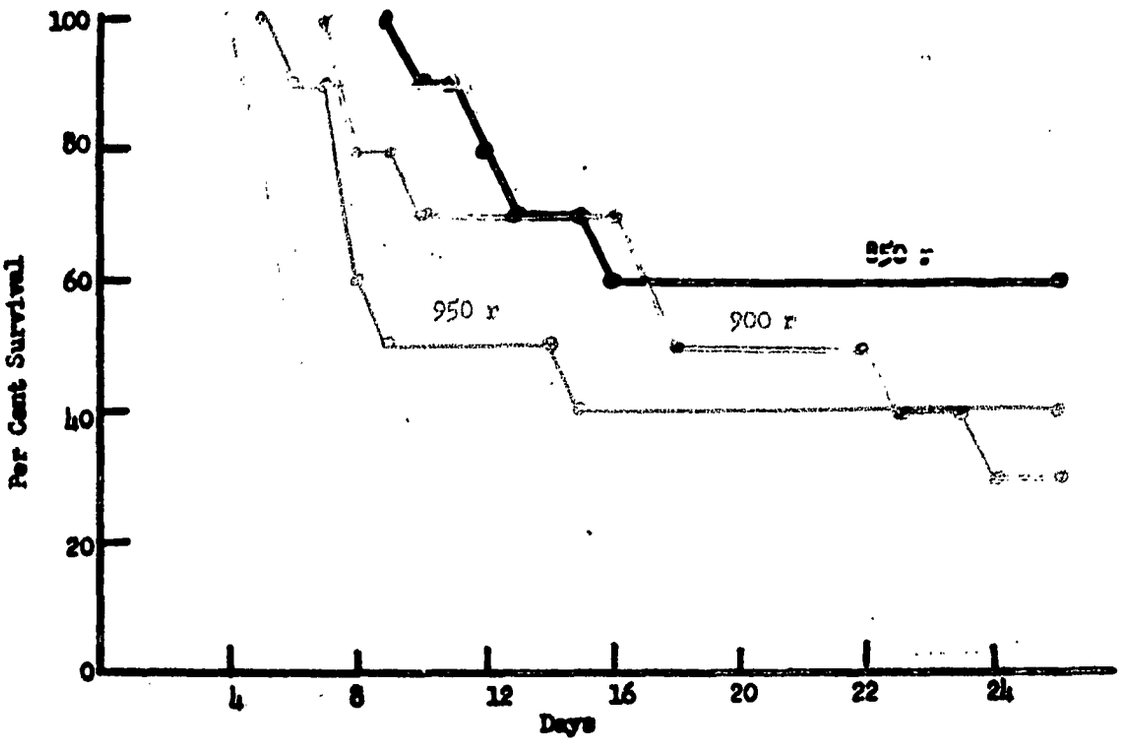

Figure 7. Survival time and mortality in CF_1 male mice given 2.50 mgm./kgm. of cyanide prior to the administration of various doses of whole-body x-irradiation.

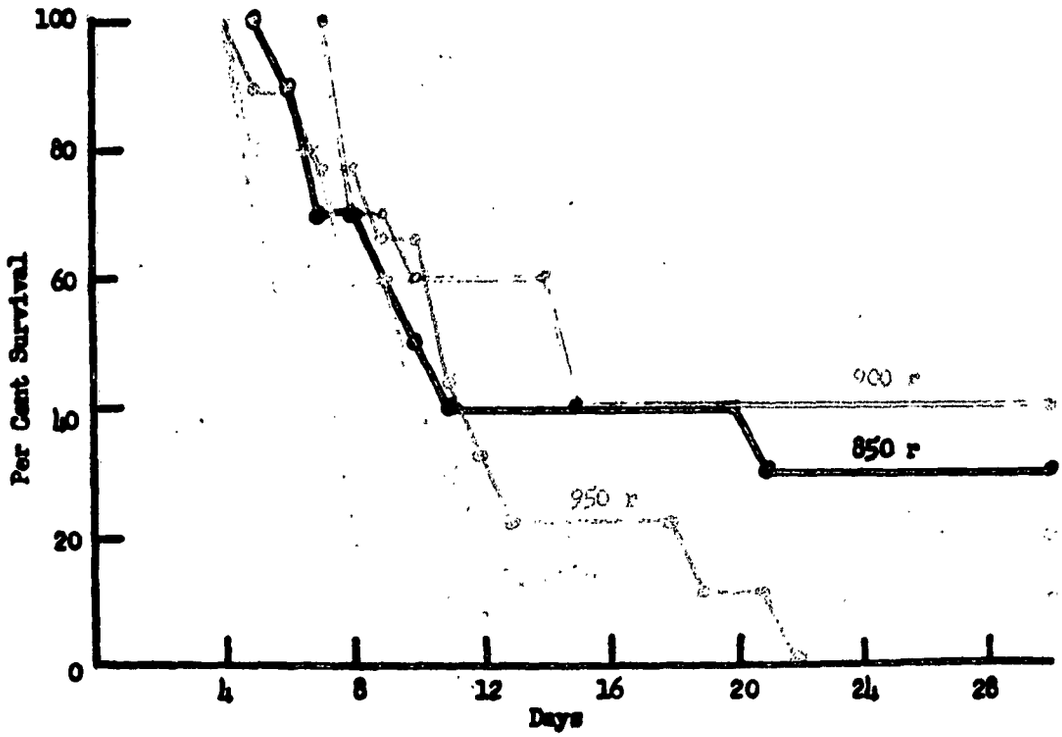

Figure 8. Survival time and mortality in CF_1 male mice given 2.75 mgm./kgm. of cyanide prior to the administration of various doses of whole-body x-irradiation.

Examination of the survival data for the male and female mice given the optimal dosage levels of cyanide in the present studies suggests that the approximate LD_{50} (30 days) for whole-body x-ray exposure in these animals is about 950 r. From other studies conducted with similar groups of animals the LD_{50} (30 days) for untreated CF_1 mice is between 450 r and 500 r. It is evident, therefore, that the dose reduction factor (DRF) for cyanide under optimal conditions approaches 2 in CF_1 mice and that its effectiveness thus is comparable with that of the best chemical radioprotective agents now available.

The effect of post-irradiation administration of 2-imino-thiazolidine-4-carboxylic acid and related compounds on the survival time of male and female x-rayed mice. Since 2-imino-thiazolidine-4-carboxylic acid has been shown (3) to be a natural metabolite of cyanide, it was of interest to investigate this compound for radioprotective activity. In previous studies (1) we found that the pre-irradiation administration of the thiazolidine derivative at a dosage level equivalent to about 50 mgm./kgm. of cyanide increased the 30-day survival of x-rayed mice from 0% to as much as 60%. The optimal time for administering the thiazolidine derivative was found to be 30 minutes prior to the radiation exposure. During these studies it also became apparent that this compound exhibited the unique capability of protecting mice against the lethal effects of radiation exposure when it was given after the exposure was completed. Subsequent studies (2) demonstrated that the interval between the end of the radiation exposure and the administration of the thiazolidine mixture was fairly critical in that protective effects were maximum at 30 minutes and decreased sharply when the derivative was given at either 1 or 60 minutes after the radiation was administered. The ability of this compound to reduce radiation lethality when given post-irradiation has been investigated further in female animals and also in male mice in the present study. Since the thiazolidine compound has not as yet been isolated and purified, the possibility cannot be excluded that the protective effects obtained with the impure material used were due in part to uncombined reactants or to the formation of other products. To investigate this possibility we have administered comparable amounts of cyanide, cystine, potassium chloride and cysteine to x-irradiated mice at various intervals following the radiation exposure. Similar studies have also been carried out with the dimer of mercaptoethylamine because of its structural similarity to both the reactants and products involved.

The effect of 2-imino-thiazolidine-4-carboxylic acid given 30 minutes after radiation exposure on the survival time and mortality of x-rayed female and male CF_1 mice is shown in Figures 9 through 12. For these studies the 2-imino-thiazolidine-4-carboxylic acid was prepared as described previously (1,2) and was given at a dosage level of 750 mgm./kgm. (assuming a complete reaction between cyanide and cystine in a 1 to 2 molar ratio) intraperitoneally. Male or female mice (16 animals per group) were exposed to each of the various radiation dosage levels and 30 minutes later half of the animals were injected with the thiazolidine mixture and the remaining mice given comparable amounts of water. The results obtained with the female mice are shown in Figures 9 and 10 and those for the male mice are shown in Figures 11 and 12. Although the thiazolidine mixture is not as protective when given after the x-ray exposure as when given prior to the irradiation, there was a significant therapeutic effect in the groups of animals given each of the radiation dosage levels in the range of 500 r through 700 r. It is evident from these studies that the post-irradiation administration of the thiazolidine mixture increased the LD_{50} for

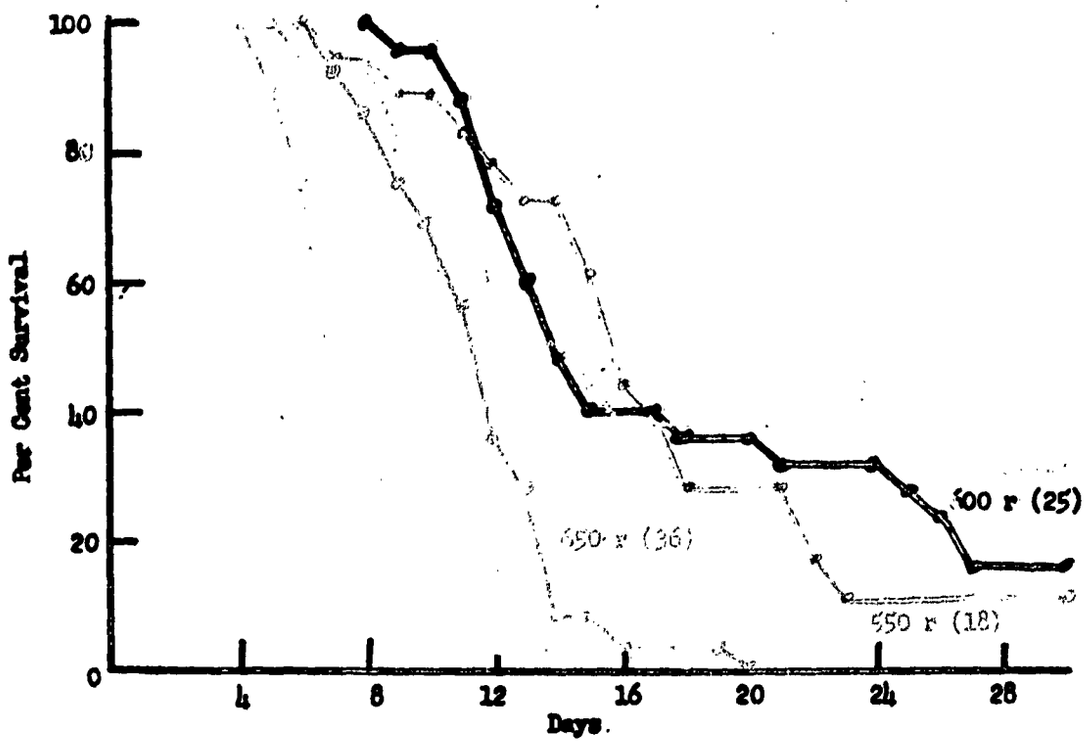

Figure 9. Survival time and mortality in CF_1 female mice exposed to various doses of whole-body x-irradiation. (The numbers in parentheses indicate the number of animals per group.)

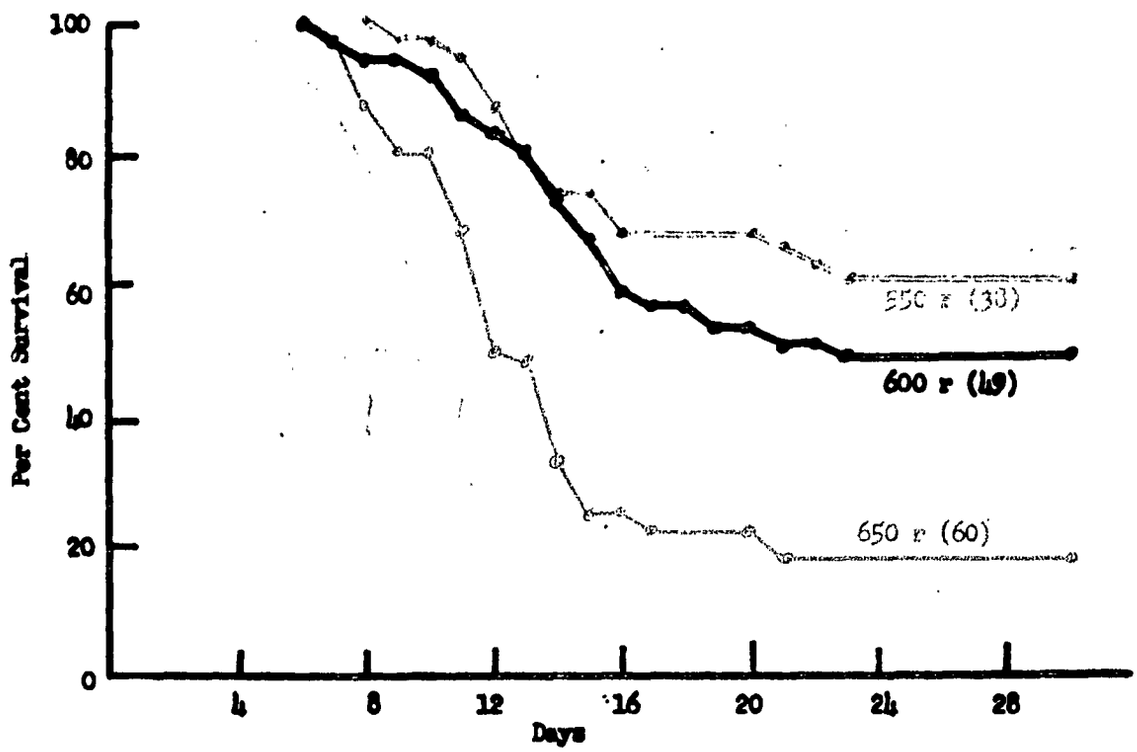

Figure 10. Survival time and mortality in CF_1 female mice given 750 mgs./kgs. of 2-imino-thiazolidine-4-carboxylic acid 30 minutes after the administration of various doses of whole-body x-irradiation. (The numbers in parentheses indicate the number of animals per group.)

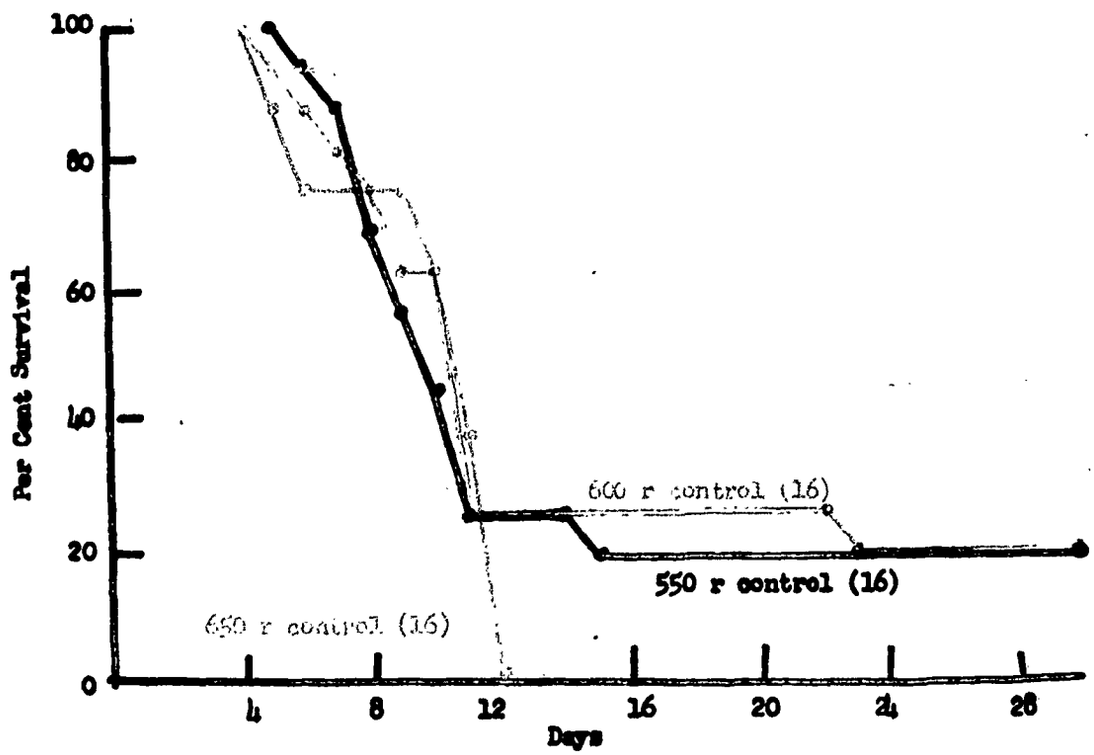

Figure 11. Survival time and mortality in CF_1 male mice exposed to various doses of whole-body x-irradiation. (The numbers in parentheses indicate the number of animals per group.)

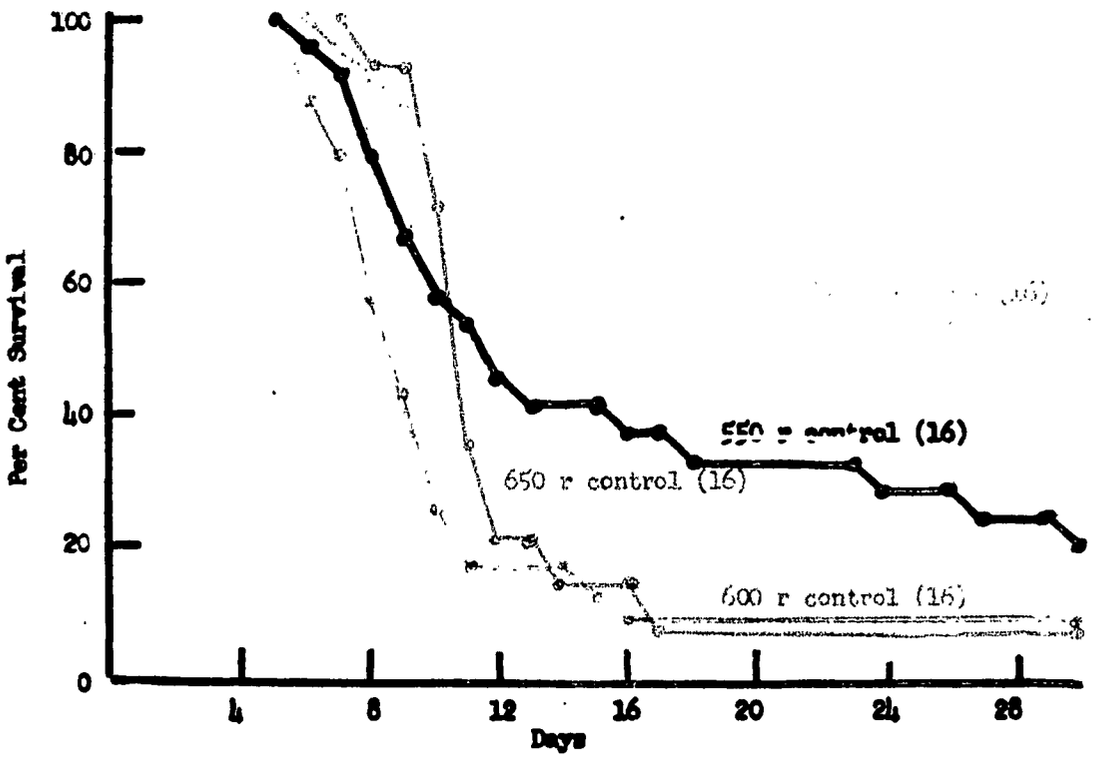

Figure 12. Survival time and mortality in CF_1 male mice given 750 mgm./kgm. of 2-imino-thiazolidine-4-carboxylic acid 30 minutes after the administration of various doses of whole-body x-irradiation. (The numbers in parentheses indicate the number of animals used in each group.)

x-rayed female mice from less than 500 r to about 600 r giving a dose reduction factor of over 1.2. In the male mice given the thiazolidine mixture, the protective effects were less evident (Figures 11 and 12). Although the number of 30-day survivors was increased by about 20% in the group of animals given 500 r and subsequently treated with the thiazolidine mixture, little or no protection was observed in the animals exposed to the higher radiation doses. The males used for these studies were large (over 25 grams) and very likely developed some anoxia during the x-ray exposure because of the confinement. Additional studies are in progress using smaller male mice and a wider spectrum of radiation dosage levels.

In previous studies in which cyanide has been used to protect mice against radiation lethality, it has been observed that when the x-ray exposure is given rapidly (600 r per minute) the symptoms of anoxia (cyanosis, respiratory depression) do not reach maximum until after the x-ray exposure is over. It was of interest, therefore, to investigate the effect of cyanide given at various intervals after the radiation exposure. Groups of eight female mice were used for these studies in which 2.5 mgm./kgm. of cyanide was administered at 1, 10, 20, 30, 40, 50, or 60 minutes after an x-ray exposure of 700 r. Two additional groups of mice were given the same dose of x-ray. One of these was given water rather than the cyanide and served as the control group and the second group was given the cyanide treatment at four minutes prior to the x-ray exposure to insure that the cyanide dosage level used in these animals was protective. The results of these studies are summarized in Figure 13. The only group of mice which exhibited 30-day survivors when the cyanide was given after the x-ray exposure was the group treated at 30 minutes after irradiation. When the cyanide was given prior to the x-ray exposure, all of the mice survived the x-ray exposure. Several of the groups which were treated at other intervals after the x-ray exposure exhibited some prolongation of the median survival time but this was rather variable and did not appear to be related to the time of administration.

In an effort to determine whether post-irradiation therapeutic effects might be obtained with any of the other reactants or products of the thiazolidine mixture, groups of eight male mice were given l-cystine (560 mgm./kgm.), l-cysteine hydrochloride (280 mgm./kgm.), potassium chloride (370 mgm./kgm.), or 2.2'-dithio-bis(ethylene amine) dihydrochloride (300 mgm./kgm.) at 30 minutes following the administration of x-ray doses of 500 r through 700 r. The results of these studies are shown in Figures 14 through 17. Since these studies were carried out at the same time as those previously described for the thiazolidine mixtures, the control animals for the thiazolidine groups were not repeated. It can be seen that none of the agents included in these tests exhibited significant therapeutic effects against radiation lethality in male CF_1 mice under the conditions of the present studies.

Discussion

The results of the studies reported here concerning the optimal dose of cyanide that affords protection at higher doses of x-ray is of interest in regard to the possible mechanisms of cyanide protection. The protective effect of cyanide is frequently considered to be due to the anoxia which it produces. If this were the sole mechanism of protection then it would seem reasonable to expect that the greater the dose of cyanide the greater would be the protection afforded.

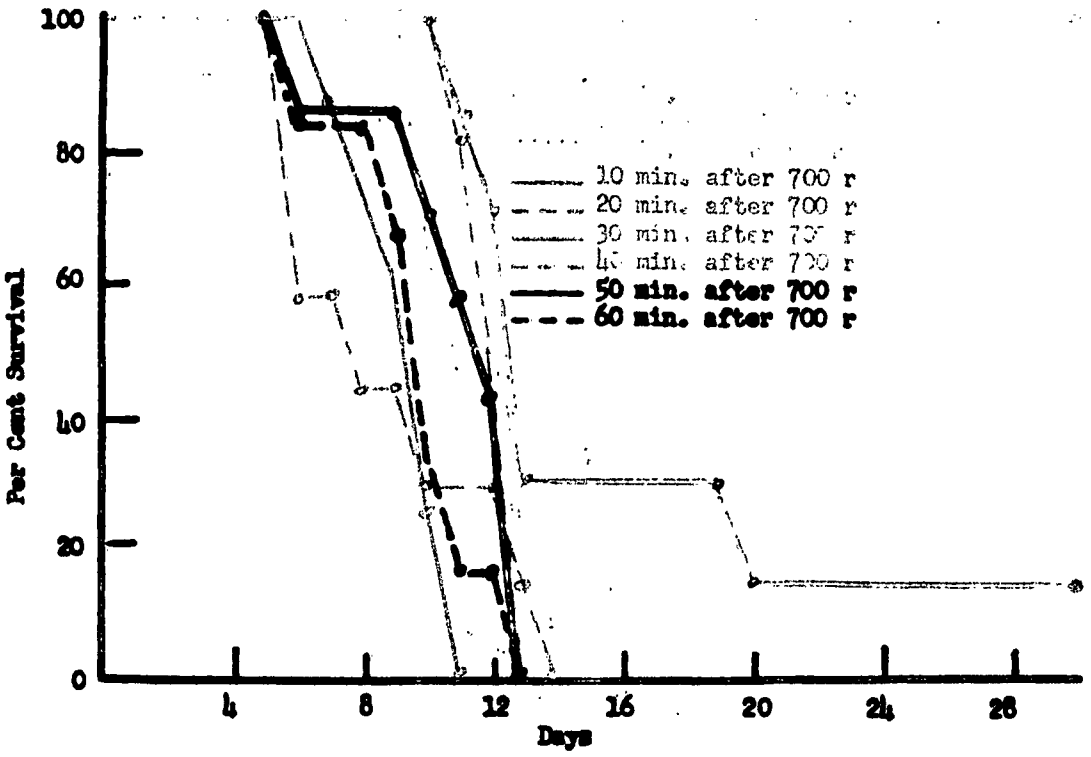

Figure 13. Survival time and mortality in CF female mice given 2.50 mgm./kgm. of cyanide at various time intervals after the administration of 700 r of whole-body x-irradiation.

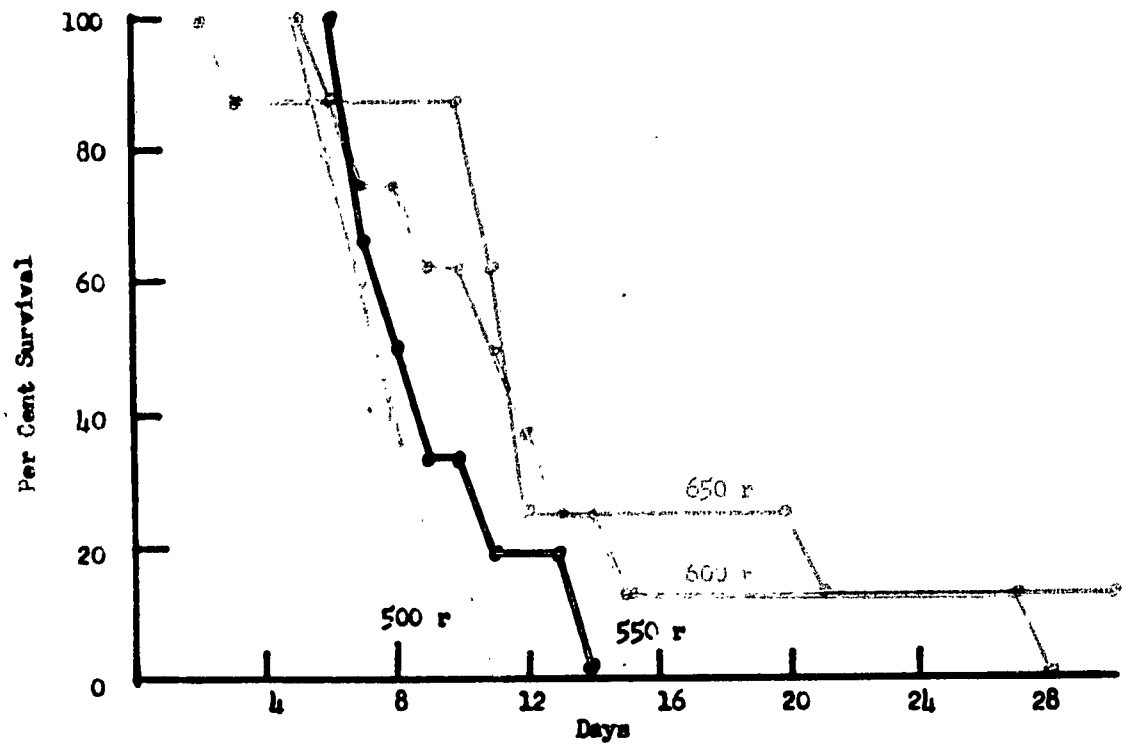

Figure 14. Survival time and mortality in CF_1 male mice given 560 mgm./kgm. of l-cystine 30 minutes after the administration of various doses of whole-body x-irradiation.

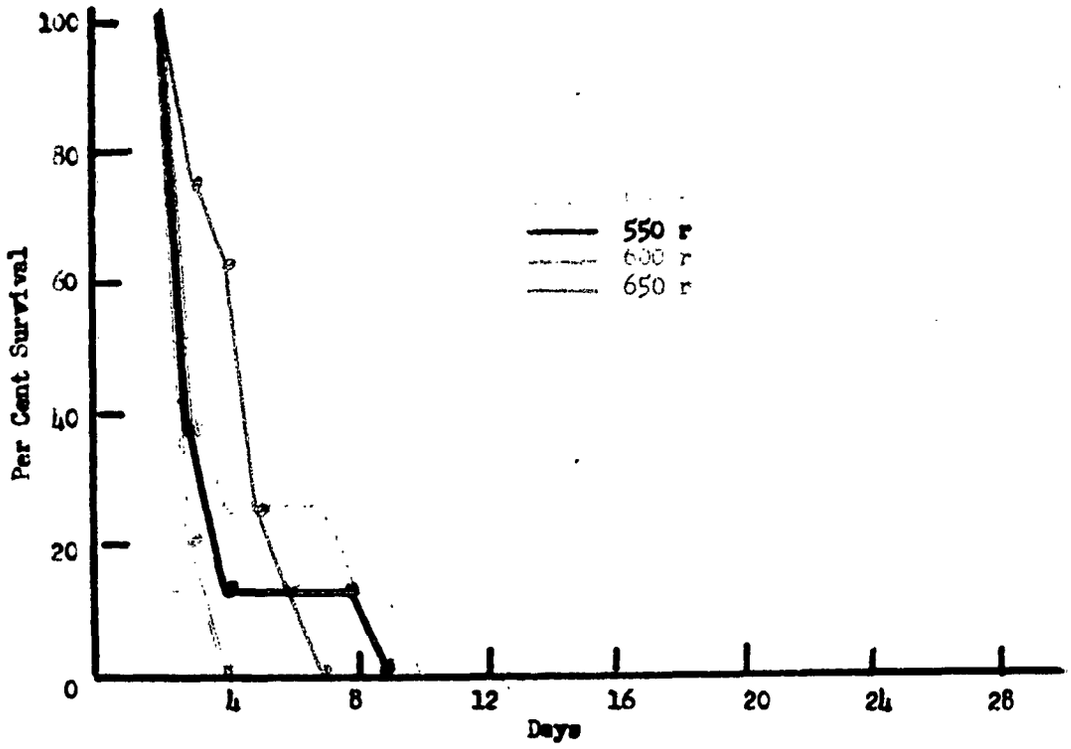

Figure 15. Survival time and mortality in CF_1 male mice given 280 mgm./kgm. of l-cysteine hydrochloride 30 minutes after the administration of various doses of whole-body x-irradiation.

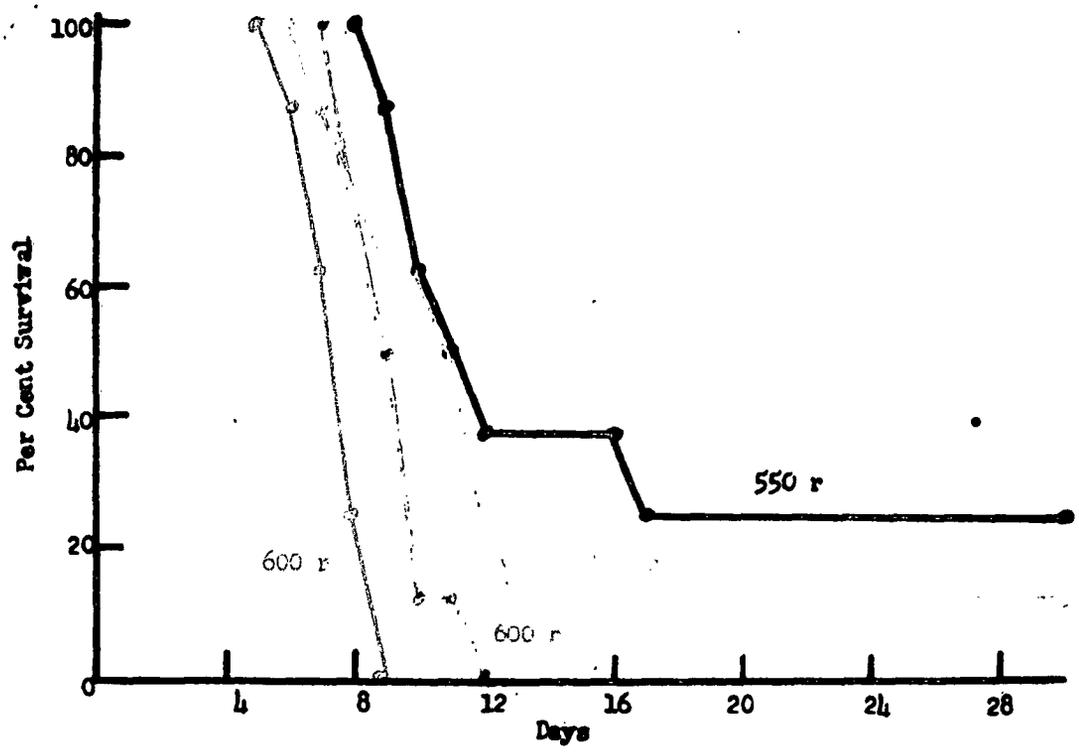

Figure 16. Survival time and mortality in CF_1 male mice given 370 mgm./kgm. KCl 30 minutes after the administration of various doses of whole-body x-irradiation.

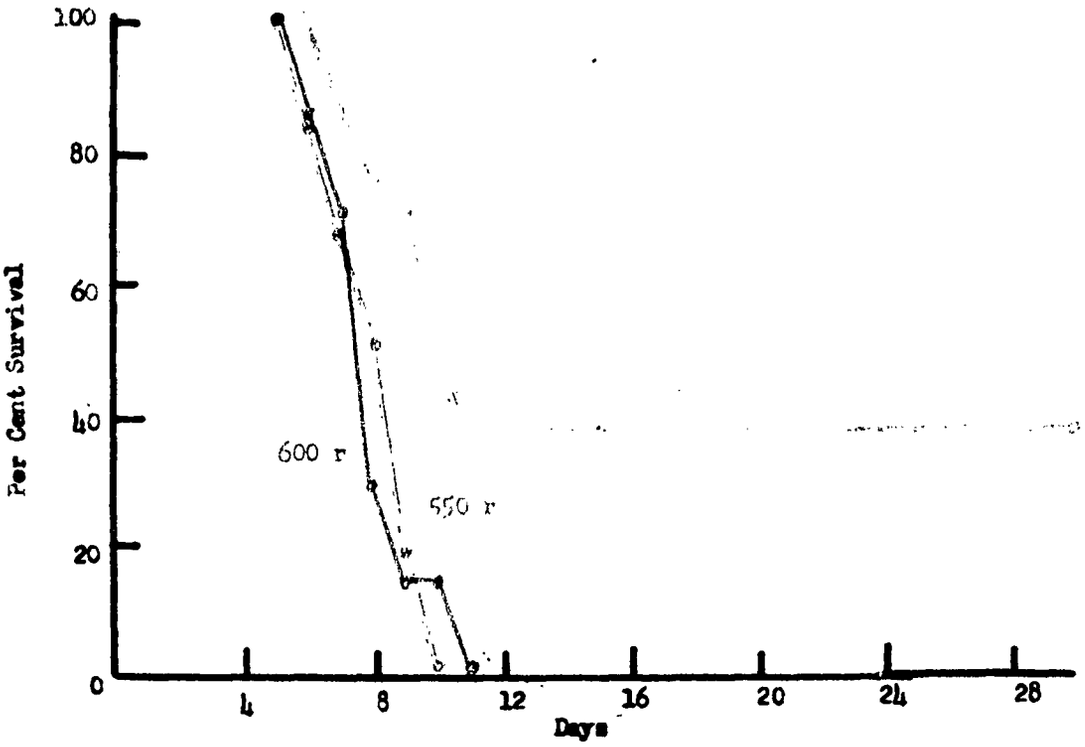

Figure 17. Survival time and mortality in CF$_1$ male mice given 300 mgm./kgm. of 2,2'-dithio-bis(ethylene amine) dihydrochloride 30 minutes after the administration of various doses of whole-body x-irradiation.

Ultimately then, one might well expect a minimum lethal dose of cyanide to be equally as protective against the lethal effects of irradiation as is the maximum tolerable amount of anoxia alone. However, in the present experiments it seems that the optimal dose of cyanide is somewhat below the minimum lethal dose and furthermore that the protective effects are partially lost at higher doses. It is not unlikely that excessive amounts of cyanide could be detrimental to experimental animals and this has, in fact, been suggested by Van Der Meer et al. (4) as a result of their studies in vitro. However, they further suggested that the protective mechanism was through the anoxia present immediately after the irradiation period. We have not been able to show this in mice which were injected immediately after the exposure to 700 r of whole-body x-ray. Further studies are planned to see if we can demonstrate any protection by producing anoxia in mice just at the end of their exposure to lethal doses of x-irradiation.

The results of the present studies support our previous finding (1) that cyanide may offer some protection when administered to mice at an interval of 30 minutes after exposure to lethal doses of whole-body x-ray. In a previous report (2) we presented evidence that 2-imino-thiazolidine-4-carboxylic acid was also effective when given 30 minutes after whole-body x-ray, and the studies presented in this report support this finding. It would seem then, that this is an effect which occurs at a rather specific time in the sequence of events following irradiation exposure which leads to the demise of the animal. This critical time period might well be responsible for much of the difficulty that others have experienced (6) in an effort to find therapeutic agents against lethal doses of radiation.

The possibility exists that the therapeutic protection obtained thus far is mostly of a palliative nature. However, the protection obtained with cyanide and 2-imino-thiazolidine-4-carboxylic acid would seem to be more chemical than biological since the time interval between irradiation and injection of the compound is so specific. Furthermore, by waiting for an additional 30 minutes all of the protective effects are lost. These findings suggest that it might be of value to investigate those enzyme systems which are responsive to the presence of cyanide or involved in its detoxification. At present we are conducting studies on the transsulfurase enzyme system, which is involved in the major detoxification of cyanide. It might also be of particular interest to again investigate those areas in the electron transport chain which are particularly sensitive to cyanide inhibition, and experiments are now planned which should enable us to do this.

In the preparation of the 2-imino-thiazolidine-4-carboxylic acid for these studies the compound was not purified before use. Therefore, other compounds were no doubt present in the injected solution. It is for this reason that we included several of these other compounds in the present test. In the original studies of this reaction Schoberl and Hamm (5) obtained a 35% yield of the calculated amount if the reaction were 100% complete. Later studies by Wood and Cooley (3) resulted in a 66% yield but with a contamination by the formation of some cysteine and in acid solution the formation of cysteine hydantoin. While our principal contaminants were probably cystine and cysteine we have now proceeded with the purification of the compound in order to verify that this is the active agent. From the results which we obtained with cystine, cysteine, and KCl it seems highly unlikely that they contributed anything to

the survival of the post-protected animals. While there may be some post-protection afforded by cyanide it is not likely that there was a sufficient quantity present because of the reduction in toxicity of the mixture. The MEA dimer was included in this set of experiments to see if the presence of a disulfide would demonstrate any protection under these experimental conditions. Further studies are planned or are in progress in an effort to determine the sensitive site(s) where the thiazolidine may be acting to produce this therapeutic effect.

Summary

1. An optimal dose of potassium cyanide equal to 2.25 mgm./kgm. of HCN and 2.50 mgm./kgm. as HCN has been found to afford maximum protection against higher doses of x-ray in female and male CF_1 mice respectively. Higher but non-lethal doses seemed to cause diminution of the protective effect.

2. An unpurified mixture of 2-imino-thiazolidine-4-carboxylic acid has been shown to protect female CF_1 mice against lethal doses of whole-body x-ray and the apparent dose reduction factor is about 1.2 based on these studies. Further studies are in progress in an effort to determine the mechanism(s) responsible for the effect.

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PHARMACOLOGICAL AND TOXICOLOGICAL COMPOUNDS AS PROTECTIVE OR THERAPEUTIC AGENTS AGAINST RADIATION INJURY IN EXPERIMENTAL ANIMALS

III. The Effect of Chemical Protection on Life-Span Shortening in X-Irradiated Mice

J. Doull, V. Plsak and J. Cowan

This report concerns: Life-span shortening and other delayed effects in female CF_1 mice given various chemical radioprotective agents prior to acute whole-body x-ray exposures of 400 r, 600 r, and 800 r.

Immediate or ultimate application of the results: Information is needed regarding the effects of chemical radioprotective agents on the delayed sequelae of acute radiation exposure. Since different radioprotective agents exhibit significant variation in their ability to prevent the various phases of the acute radiation syndrome, it is likely that they also exhibit differences in their ability to prevent the various delayed effects of such exposure. A better understanding of the capability of each agent to protect against both the acute and delayed aspects of radiation injury should be of value in the selection of agents for further investigation and should contribute substantially to progress in elucidating the mechanisms responsible for these effects.

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Virtually all of the extensive effort which has been devoted to the development of chemical radioprotective agents during the last decade has been concerned with preventing radiation injury during the immediate post-irradiation period. From these studies it is now apparent that there are both qualitative and quantitative differences in the response of the various mammalian systems in animals given radioprotective agents. Although these differences may be due in part to variation in the penetrability of the agents into radiosensitive sites, it seems likely that these differences are more closely correlated with the variations in the mode of action of the specific radioprotective agents. The investigation of the ability of various radioprotective agents to reduce radiation injury to diverse mammalian systems is of value, therefore, in determining not only how these systems contribute to the radiation syndrome but also why some radioprotective agents are more effective than others against specific radiation dosage levels. Serotonin (5-HT) and mercaptoethylamine (MEA), for example, are both capable of markedly reducing radiation lethality in x-rayed mice and both minimize radiation injury in the hematopoietic system of such animals. 5-HT is far less effective than MEA, however, in preventing radiation injury to the gastrointestinal tract and its ability to reduce radiation lethality in mice given supralethal doses of radiation is, therefore, less than that of MEA. Similar differences exist in the ability of the chemical radioprotective agents to prevent oral radiation death or injury to the central nervous system. Less is known about the ability of the chemical

radioprotective agents to prevent the delayed or late effects of acute radiation exposure. Such agents have been reported to protect against radiation-induced cataractogenesis (1) and graying of hair (2) but not against the genetic effects, neoplasia and premature aging from such exposures (3-6). The present studies were carried out in an effort to obtain more detailed information concerning the ability of the chemical radioprotective agents to prevent the delayed effects of acute x-ray exposure. Groups of female mice have been given radioprotective agents at dosage levels adequate to markedly reduce acute radiation lethality and the mortality of these mice was followed during a period of about one year after radiation exposures of 400 r through 800 r. Many of the animals which died during the course of these studies have been autopsied and the tissues prepared for histological examination. In addition mice from each of the groups have been sacrificed at various intervals following the x-ray exposures in order that information concerning the time of onset of the various lesions might be obtained. The present report contains the results of the mortality observations made during the first year after the x-ray exposure and the gross and microscopic pathology for these mice will be reported subsequently.

Materials and Methods. Adult, female Carworth Farms CF_1 mice between 9 and 15 weeks of age were used for these studies. The animals were randomized and then divided into five groups each of which contained four sub-groups of 32 mice. Four of the five groups were given one of the radioprotective agents and the fifth group (radiation controls) was given comparable amounts of water and irradiated simultaneously with the protected animals. The x-ray exposures were given 15 minutes after the administration of the protective agents and each of the sub-groups received 0 r (drug controls), 400 r, 600 r, or 800 r of whole-body x-irradiation. The radiation dose in the non-protected groups given 600 r and 800 r was fractionated (200 r every other day) to avoid excessive mortality. The mortality in each group was observed daily during the first 30 days after the x-ray exposures and at weekly intervals thereafter. The animals were housed in stainless steel cages and raised wire screen floors (eight per cage) in an air-conditioned room (80° F. ± 3° F.) and were provided with food (Rockland Mouse Food) and water ad libitum.

The following radioprotective agents were included in these studies: 2-mercaptoethylamine hydrochloride (CB-4451, MEA), p-aminopropiophenone (UCTL-1856, PAPP), serotonin creatinine sulfate (CB-32, 5-HT), and 2-aminoethylisothiourea dichloride (B-53, AET). All of the compounds were dissolved in water except for PAPP which was dissolved in 50% propylene glycol. The injection solutions were prepared freshly and the concentration was adjusted so that the mice received not more than 1% of their body weight with each intraperitoneal injection. The dosage levels used for these studies are shown in Table 1.

The x-ray exposures were given by means of a G. E. Maximar X-ray unit operated at 250 KVP and 15 ma. with 0.25 mm. copper and 1.0 mm. aluminum added filtration (HVL = 1.01 mm. copper). The dose rate was determined at the beginning and end of each radiation exposure by means of a Victoreen Ionization Chamber which was placed in an empty mouse holder located in an appropriate position. In addition the dose rate was monitored during the radiation exposure by means of a Victoreen Roentgen Ratemeter (Model 510) provided with a median energy probe assembly (Model 601). The control and protected animals were irradiated together in groups of 16 mice contained in individual lucite tubes (50 cc. centrifuge tubes provided with numerous air-holes) which were

placed perpendicularly to the central axis of the x-ray beam on a rotating turntable. The environmental temperature of the mice was thermostatically controlled during the x-ray exposures (80 °F. ± 2° F.).

TABLE 1

NUMBER OF MICE AND DOSAGE LEVELS OF RADIOPROTECTIVE AGENTS EMPLOYED

Group No.	Protective Agent and Dosage Level	Number of Mice Surviving at 30 Days After Exposure			
		0 r (Drug Controls)	400 r	600 r	800 r
1	2-Mercaptoethylamine hydrochloride 300 mgm./kgm. IP	32/32	29/29	31/32	26/30
2	p-Aminopropiophenone 30 mgm./kgm. IP	31/32	31/32	30/30	29/32
3	Serotonin creatinine sulfate complex 90 mgm./kgm. IP	32/32	32/32	32/32	23/32
4	2-Aminoethylisothiourea dichloride 200 mgm./kgm. IP	32/32	32/32	30/32	27/32
5	Radiation controls (vehicle only)	32/32	30/32	30/38	21/42

Results

Life-span shortening in female CF_1 mice surviving the acute (30-day) effects of whole-body x-ray exposure. The mortality in the groups of mice exposed to 400 r, 600 r, and 800 r of x-irradiation has now been followed for a period of over 60 weeks and the results of these observations are summarized in Figure 1. Since the present studies are concerned only with the effect of the chemical protectors on life-span shortening, the per cent mortality in each group has been computed on the basis of the number of mice which were surviving at the end of the first 30-day period after the x-ray exposure. It can be seen in Table 1 that there was an appreciable mortality in the non-protected groups of mice given 600 r and 800 r even though the x-ray dose was fractionated and administered over a period of about a week. Thus the two highest dosage level groups of the non-protected mice represent a selected population and this is

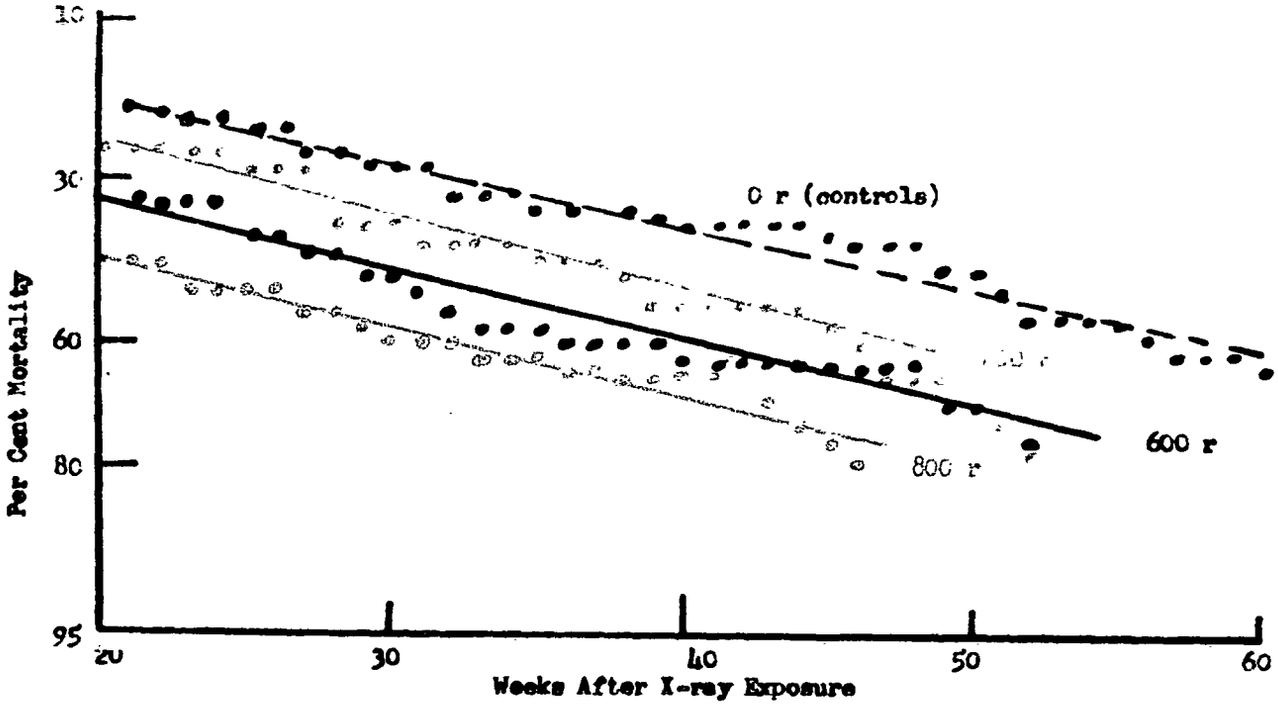

Figure 1. Longevity in female mice surviving the acute lethal effects of whole-body x-ray exposures of 400 r, 600 r, and 800 r.

also true to a lesser extent for some of the protected groups of animals. The mortality data shown in Figure 1 covers only the period from 20 to 60 weeks after the x-ray exposure, since this period included the median survival time for all of the groups for which we have sufficient data at the present time. It can be seen in Figure 1 that the median survival time (ST_{50}) for the control group (non-protected, non-irradiated) was about 50 weeks which would correspond to about 62 weeks as the actual average age of the mice at the time when 50% of the group had died. The age corrected median survival times for the mice exposed to 400 r, 600 r, and 800 r were found to be about 52, 45, and 36 weeks respectively. Thus the life-span shortening for the non-protected mice given 400 r, 600 r, and 800 r would be approximately 16%, 28%, and 42% respectively. It would appear, therefore, that the life expectancy of these animals has been decreased by about 5% for each 100 r of x-irradiation which they received.

Effect of serotonin creatinine sulfate (5-HT) on the life-span shortening effect of acute whole-body x-ray exposure in CF_1 female mice. The mortality data for the groups of mice which were treated with 5-HT prior to the x-ray exposures is shown in Figure 2. The control group of mice shown in this figure were given 5-HT but were not irradiated. It is evident that the median survival time of the mice treated with the 5-HT is prolonged significantly over that of unirradiated, non-treated control animals (age corrected ST_{50} of 72 weeks as compared with the previous value of 62 weeks). The corrected ST_{50} values for the mice given 5-HT prior to the administration of 400 r, 600 r, and 800 r were found to be about 60, 55, and 44 weeks respectively. If the life-span shortening is computed on the basis of the ST_{50} of the 5-HT treated control group, one obtains values of about 17%, 22%, and 39% for the groups of mice exposed to x-ray doses of 400 r, 600 r, and 800 r respectively. Calculation of the life-span shortening in terms of the ST_{50} of the non-protected control group of mice, however, reduces the values to about 3%, 10%, and 29% for the x-ray dosage levels of 400 r, 600 r, and 800 r respectively.

Effect of p-aminopropiophenone (PAPP) on the life-span shortening effect of acute whole-body x-ray exposure in CF_1 female mice. In Figure 3 is shown the mortality data for the mice which were given 30 mgm./kgm. of PAPP prior to the x-ray exposures. The drug control group (PAPP only) exhibited an increased median survival time when compared to that of the non-treated controls. The age corrected median survival time for the group of mice which were given the PAPP treatment prior to an x-ray exposure of 400 r was 58 weeks and the corresponding values for the mice exposed to x-ray doses of 600 r and 800 r are 46 and 44 weeks respectively. Comparison of the survival time of these groups with that of the untreated control group gives life-span shortening values of 6%, 26%, and 29% for the 400 r, 600 r, and 800 r groups. Comparison of these values with the PAPP control group (age corrected median survival time of 73 weeks) results in life-span shortening values of 20%, 37%, and 40% for the three x-ray exposures.

Effect of 2-aminoethylisothiourea dichloride (AET) on the life-span shortening effect of acute whole-body x-ray exposure in female CF_1 mice. The mortality data for the groups of mice treated with AET prior to the x-ray exposures is shown in Figure 4. The age corrected median survival time for the non-irradiated mice given the AET was estimated to be about 78 weeks and that for the animals given the AET prior to the x-ray exposures was 59, 48, and 36

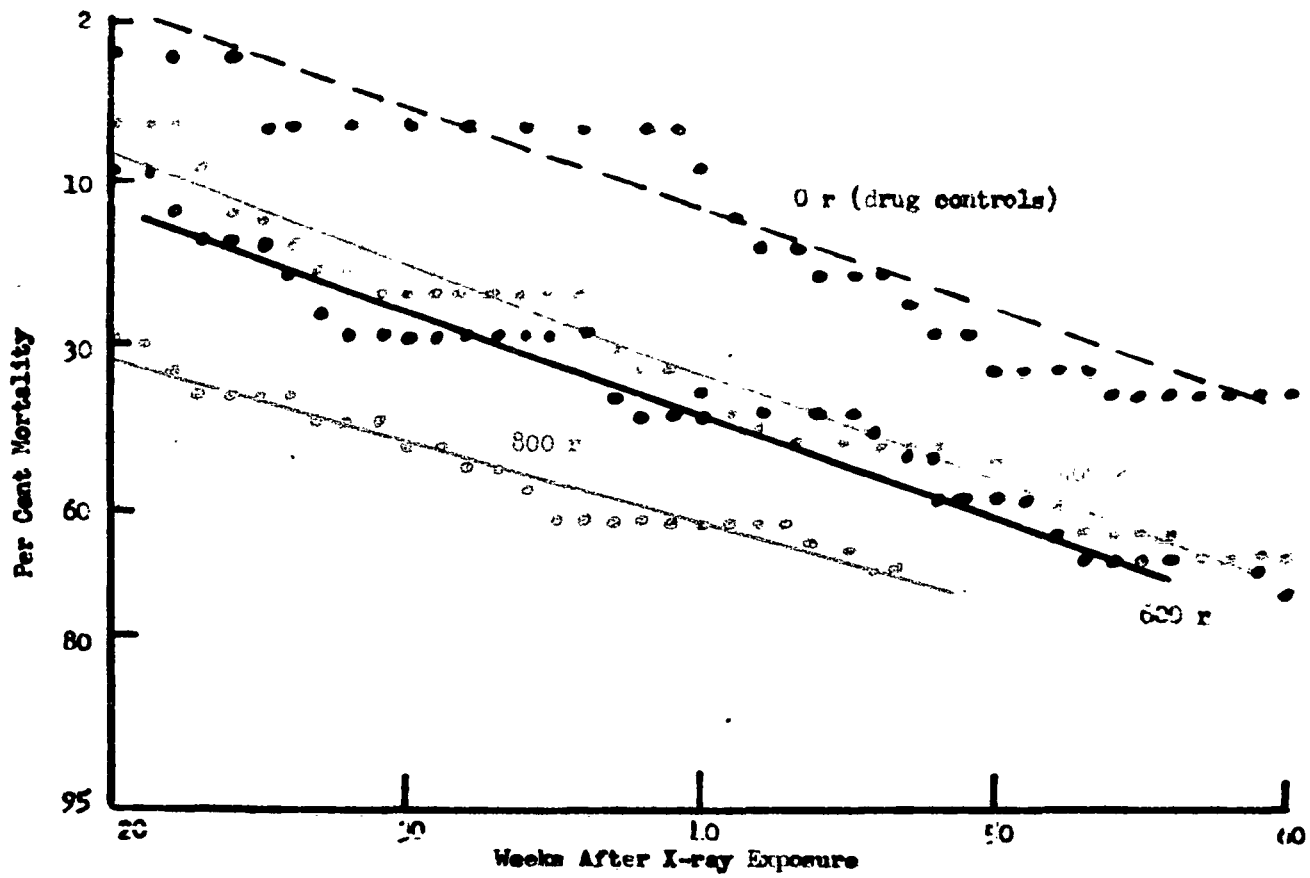

Figure 2. Effect of serotonin creatinine sulfate (5-HT) on the life-span shortening effect of acute whole-body x-ray exposure in CF_1 female mice.

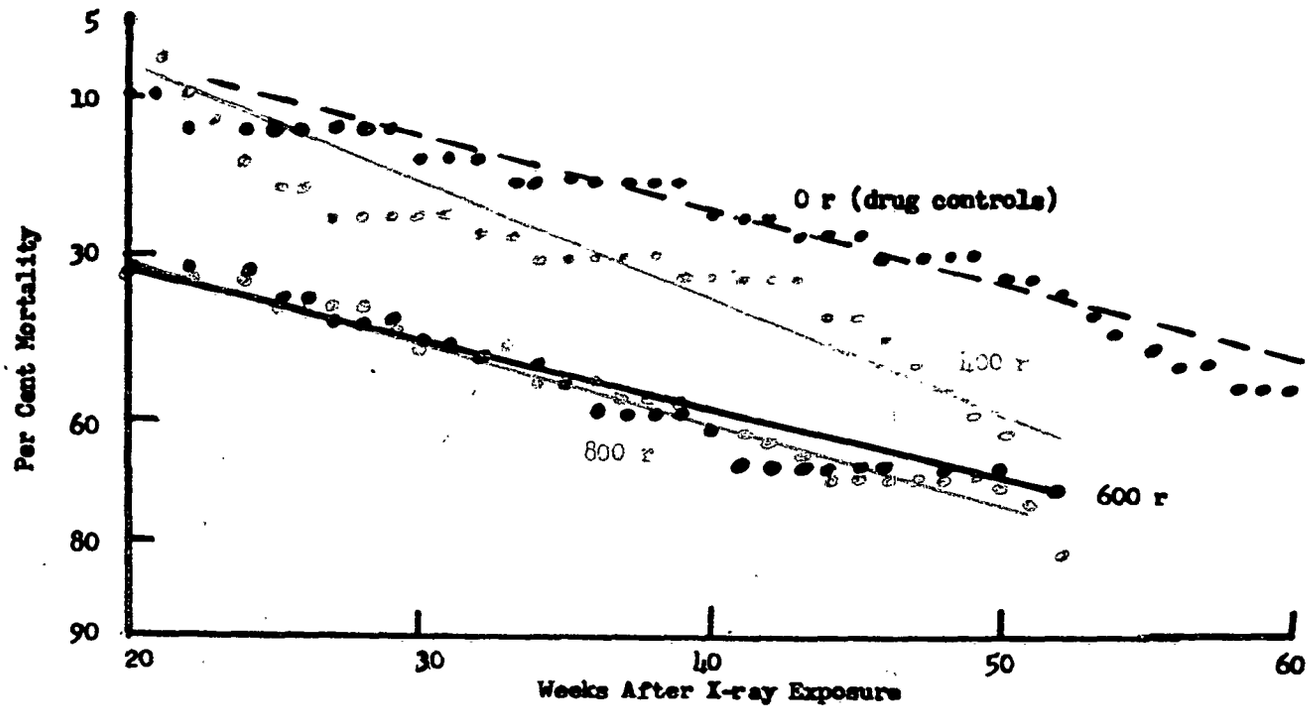

Figure 3. Effect of p-aminopropiophenone (PAPP) on the life-span shortening effect of acute whole-body x-ray exposure in CF_1 female mice.

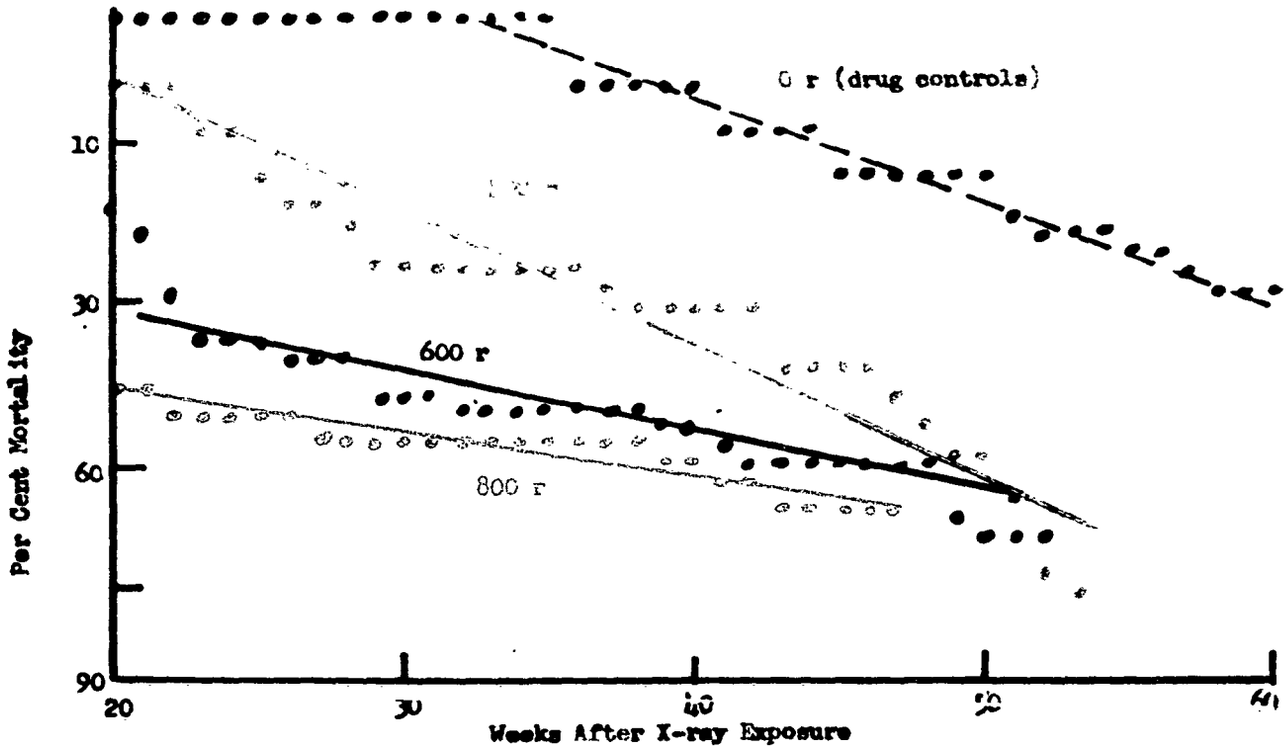

Figure 4. Effect of 2-aminoethylisothiourea dichloride (AET) on the life-span shortening effect of acute whole-body x-ray exposures in female mice.

weeks for the groups exposed to 400 r, 600 r, and 800 r respectively. The life-span shortening in these animals was about 5%, 23%, and 42% when compared to the survival time of the non-irradiated untreated controls and about 24%, 37%, and 54% when compared to the ST_{50} of the AET-treated control group.

Effect of 2-mercaptoethylamine hydrochloride (MEA) on the life-span shortening effect of acute whole-body x-ray exposure in CF_1 female mice. The mortality data for the MEA-treated groups of animals is presented in Figure 5. Since the mortality in the control group (MEA only) has not yet reached 30%, it is not possible to estimate the median survival time for these mice but it is evident from the results obtained thus far that the value will probably be greater than 80 weeks (corrected for age). The mice which were given MEA prior to the 400 r x-ray exposure exhibited an age corrected median survival time of about 53 weeks and the group of animals which received 800 r of x-irradiation following the MEA treatment had a median survival time of about 38 weeks. The value for the group of mice exposed to 600 r has not been included in Figure 3 since several of these animals died during the early part of the observation period and it will be necessary to repeat the studies at this dosage level. From these studies the life-span shortening in the mice exposed to the x-ray dosage levels of 400 r and 800 r was 14% and 39% on the basis of the untreated control group.

Discussion

The evaluation of radioprotective agents in terms of their ability to reduce the effective radiation dose is meaningful only when it is recognized that such dose reduction factors (DRF's) are both time and dose dependent. The DRF value for radioprotective chemicals is most frequently determined by the ability of the agent to increase the 30-day LD_{50} value in animals exposed to whole-body x-ray doses in the range of 400 r through about 1,400 r. Since the major cause of radiation death in this dosage range and time period is hematopoietic injury, computation of the DRF on the basis of the 30-day mortality data provides a value which reflects primarily the ability of the protective agent to prevent injury to the hematopoietic tissues. Previous studies in this laboratory (7) have shown that the determination of the DRF for serotonin at six days after radiation was about 1.1 whereas the same animals exhibit a DRF value of about 1.8 when the computations are based on the mortality data 30 days after the x-ray exposure. Thus by computing the DRF values for a specific protector at various intervals following the radiation exposure, one can obtain information concerning the ability of the protector to decrease damage in the tissue or system which is contributing most heavily to mortality during each interval following the radiation exposure. In attempting to determine whether the chemical radioprotective agents are as effective in preventing the delayed effects of radiation exposure as they are against the acute radiation sequelae, it would be useful to be able to compare DRF values for the two situations. While the range of x-ray dosage levels used for the present studies is inadequate for this type of approach, it is possible to obtain an indication of the effects of the agents by comparing the life-span shortening in the protected and non-protected groups of mice.

In Figure 6, the life-span shortening data from the non-protected groups of mice has been expressed in terms of the x-ray dose. Although the results obtained in the present study suggest that there is a threshold at about 150 r,

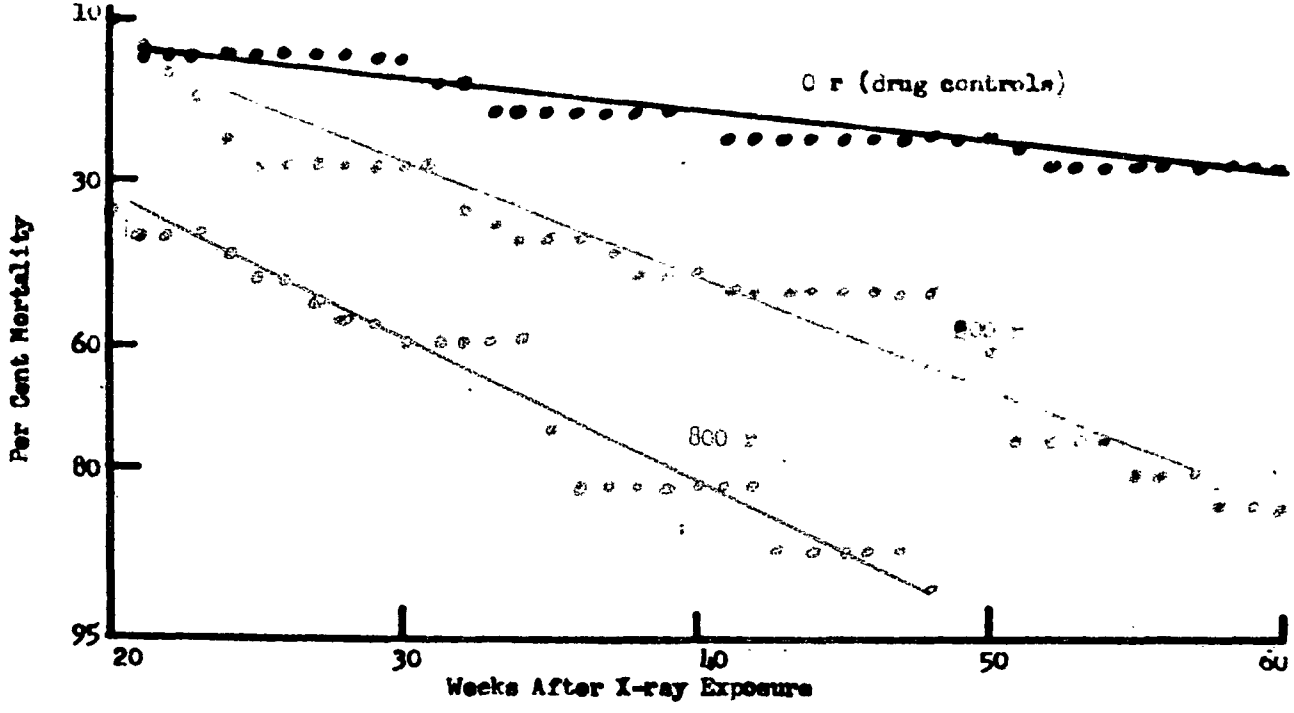

Figure 5. Effect of 2-mercaptoethylamine hydrochloride (MEA) on the life-span shortening effect of acute whole-body x-ray exposure in CF_1 female mice.

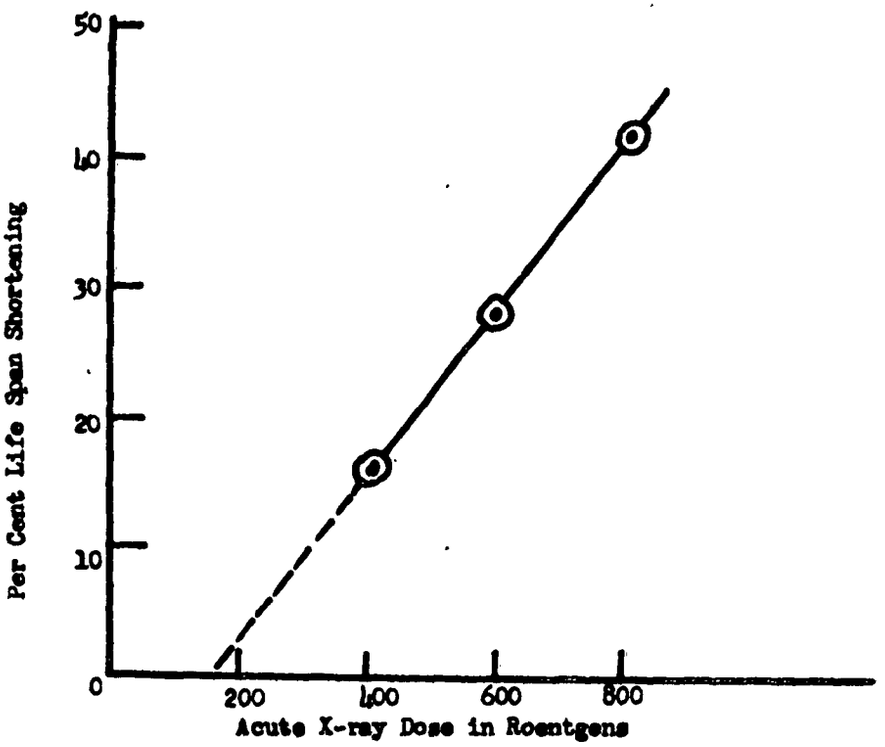

Figure 6. Life-span shortening in CF_1 female mice as a function of the acute x-ray dosage level.

comparable studies from other laboratories (8,9) in which wider ranges of doses were used suggest that the threshold is lower if it does in fact exist. If a linear relationship is assumed to exist for the per cent life-span shortening in terms of the radiation dose, the results of the present study would suggest that the life span of CF_1 female mice is decreased by about 0.047% per roentgen of acute x-ray exposure. On the basis of this value the per cent life-span shortening observed in each of the protected groups of mice can be used to calculate the effective dose of radiation which each of the groups received and this in turn permits calculation of an approximate DRF value for each protected group. Such calculations are complicated in the present studies by the increased survival of the non-irradiated groups treated with each of the protective agents. Since the mortality in these groups has not yet reached 50%, the median survival time of these groups must be estimated on the basis of limited mortality data particularly for the groups treated with MEA. The results of these calculations are summarized in Table 2 where the data for the MEA-treated mice has been computed on the basis of an assumed median survival time of 82 weeks for the non-irradiated controls. It is evident from an examination of the age corrected survival times that none of the agents tested in these studies were of particular benefit in preventing the life-span shortening effect of the x-ray exposures. The median survival time of the mice treated with 5-HT, PAPP, and AET prior to an x-ray exposure was increased over that of the non-protected animals by about nine weeks and that of the mice given 5-HT or PAPP before the 800 r x-ray exposure was increased by about eight weeks. In the mice exposed to the x-ray dose of 600 r the only agent which increased the median survival time was 5-HT. Correction of these data for the prolonged survival time of the non-irradiated control groups, however, eliminates the protective effect for all of the groups except those treated with 5-HT and given 400 r or 600 r and the mice given AET prior to 400 r of x-ray. The average DRF for the 5-HT treated animals is about 1.1 and the values for the mice given PAPP, AET, and MEA are about 0.88, 0.92, and 0.64 respectively. The DRF values for these agents against acute radiation lethality (30-day survival) are all above 1.5 and it is evident, therefore, that none of the chemical radioprotective agents included in these studies, with the possible exception of 5-HT, are effective in preventing the life-span shortening effects of acute x-ray exposure in CF_1 female mice. In the present studies pre-treatment with MEA and AET actually decreased the life-span of the x-rayed mice.

The results obtained in these studies are similar to those reported by Maisin et al. (10) who found that pre-treatment with MEA did not reduce the life-span shortening effect of 600 r in rats. In these studies the non-irradiated controls survived for about 18 months and the x-ray exposure reduced the median survival time by about six months irrespective of whether the animals were given MEA or water prior to the x-ray exposure. The studies of Mewissen and Brucer (6) suggest that neither MEA nor the MEA dimer were capable of preventing the life-span shortening effects of whole-body exposure to Co^{60} gamma irradiation at dosage levels of 406 r or 550 r. Holleroft et al. (1) have reported that spleen shielding or the administration of cysteine plus hypoxia did not extend the life-span of C3H mice given 400 r of x-irradiation. However, Upton et al. (12) noted an increased median survival time in partially shielded mice exposed to 450 r of x-irradiation. Mercaptoethylguanidine (the active form of AET) has been reported (13) to decrease not only the life-span shortening effect of x-ray doses in the range of 800 r through 1,400 r but also the tumor incidence. In these studies there was no evidence that the protective agent decreased cataract

TABLE 2

LIFE-SPAN SHORTENING IN CF_1 FEMALE MICE TREATED WITH CHEMICAL RADIOPROTECTIVE AGENTS PRIOR TO ACUTE X-RAY EXPOSURE

Radioprotective Agent	Acute X-ray Dose in Roentgens			
	0 r	400 r	600 r	800 r
5-HT	72	60[a] 360[b] 1.10[c]	55 510 1.18	44 810 0.99
PAPP	73	58 425 0.94	46 790 0.76	44 850 0.94
AET	78	59 300 1.31	48 810 0.74	36 1150 0.70
MEA	> 80	53 720 0.55	..	38 1100 0.73
None	62	52 362	45 595	36 880

[a]Age corrected median survival time in weeks.

[b]X-ray dose in r corresponding to life-span shortening effect.

[c]DRF (effective dose in r/administered dose in r).

formation whereas Von Sallmann has reported (14) that cysteine reduces cataractogenesis in rabbits given 1,500 r to the eyes. The only similar studies carried out with PAPP appear to be those of Brecher et al. (5) who found that young rats given lethal doses of x-irradiation exhibited a shortened survival time and increased tumor incidence although it is difficult to evaluate these studies because of the lack of proper control groups. In most of the studies which have been carried out, the life-span shortening effect in the protected-irradiated groups has been compared with the life-span shortening effect in non-protected animals exposed to the same radiation dose. The results of the present studies demonstrate that such groups do not constitute an adequate control since the protective agents may also influence longevity. If the results of the present study had been evaluated on the basis of the non-protected control group, it would be difficult to avoid the conclusion that both 5-HT and PAPP were effective in preventing the life-span shortening effects of radiation exposure. It is apparent that additional studies are indicated and that these studies should include a wider range of radiation dosage levels and a larger number of animals particularly in the control groups. The question of whether the chemical radioprotective agents prevent the delayed sequalae of acute radiation exposure is, of course, only partially answered by the results of these studies. Cataracts and gross tumors were not observed in any of the mice included in these studies. However, the histopathological examination of the tissues from these mice which is in progress should provide a comparative estimation of the effects of the protectors on tumor formation.

Summary

1. The longevity of female CF_1 mice given various chemical radioprotective agents prior to acute x-ray exposures of 400 r, 600 r, and 800 r has been compared with that of non-irradiated animals given the protectors and with non-protected irradiated animals in order to determine whether any of these agents reduce or prevent the life-span shortening effects of acute x-ray exposure.

2. Pretreatment with serotonin creatinine sulfate (90 mgm./kgm. IP) increased the age corrected median survival time of the x-rayed mice by about eight weeks but the protective effect is small in comparison with the ability of this agent to prevent acute (30-day) radiation effects.

3. Mice pretreated with p-aminopropiophenone (30 mgm./kgm. IP), 2-aminoethyl-isothiourea dichloride (200 mgm./kgm. IP), or 2-mercaptoethylamine hydrochloride (300 mgm./kgm. IP) prior to the administration of x-ray doses of 600 r or 800 r exhibited age corrected median survival times comparable or less than those seen in animals given these x-ray doses without protection. All of these agents increased the median survival time of mice given 400 r of x-irradiation but correction of the life-span shortening data for the increase in longevity produced by the administration of the agents alone eliminated the apparent protective effects in these animals.

4. No evidence was obtained in the present studies that pretreatment with the radioprotective agents decreased the incidence of other delayed radiation sequelae.

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PHARMACOLOGICAL AND TOXICOLOGICAL COMPOUNDS AS PROTECTIVE OR THERAPEUTIC AGENTS AGAINST RADIATION INJURY IN EXPERIMENTAL ANIMALS

IV. Effect of Various Radioprotection Treatments in Mice

H. D. Landahl and A. T. Hasegawa

This report concerns: Experiments on protection against the injurious effects of x-irradiation by external spleen shielding in conjunction with serotonin (5-hydroxytryptamine) and MEA (mercaptoethylamine). In other experiments hydroxylamine, low oxygen tension, sodium cyanide, or sodium nitrite plus MEA were used to enhance protection. The effect of topical application of histamine or AET (aminoethylisothiouronium dichloride) to the exteriorized spleen as well as spleen shock before irradiation was also studied.

Immediate or ultimate application of the results: To obtain information concerning the mechanisms involved in radioprotection by treating animals in various ways, both chemical and physical, prior to x-irradiation.

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In the first series of experiments presented in this report the LD_{50} of x-irradiation to mice was determined under conditions in which the spleen is protected by an external lead shield during irradiation. Using this value as a base line, the protection by any compound over and above that provided by the shielding alone may be attributed to protection of other systems. With this point of view in mind, the effects of MEA and of serotonin were investigated.

In the second series of experiments an attempt was made to determine the extent of the increase in protection which can be achieved by increasing the amount of protective substance, which was hydroxylamine in this case. According to the scavenger hypothesis one would expect that increasing amounts of a protective substance would result in increasing protection and this protection could conceivably be large, but the amount of the substance that can be given is limited by its toxicity. In the case of protection by decreasing oxygen, the damage which is mediated through oxygen might decrease to a very low level but such a level cannot be determined if it is below that level which can just be tolerated.

Thirdly, an experiment was carried out to see whether the protection by sodium cyanide correlated in time with the decrease in spleen oxygen tension. The fourth series of experiments consisted of determinations of the effectiveness of topical application of compounds and of electric shock to the spleen in an attempt to protect the animals against the lethal effects of irradiation by protecting the spleen alone. Lastly, an experiment was performed to ascertain whether there was additive protection when MEA and sodium nitrite were administered before irradiation.

Materials and Methods. Adult CF_1 male mice weighing between 22 and 28 grams were used for these experiments. They were maintained on Rockland Mouse Pellets and water ad libitum. The mice were irradiated two at a time in a small circular plastic chamber divided so that only one mouse occupied one side. The chamber was constructed so that air or mixtures of gases could flow through it to maintain any desired environmental oxygen tension. The irradiation was given at a rate of 600 r per minute with a Maximar G.E. unit using 250 KVP, 15 ma. with 0.25 mm. Cu and 1 mm. Al added filtration.

Results

Protection against irradiation by an external shield over the spleen. As a reference point for comparison of protection against high levels of irradiation, a dose reduction factor for an external spleen shield is desirable. Nembutalized mice were placed in a small chamber on their right sides and a small 1/4" wedge of lead was placed over the spleen with the lead resting on the animal. The wedge was 2 cm. long and 0.7 cm. wide at the end projecting over the abdomen, and 1 cm. wide at the end which was directly over the backbone. About 30 minutes after being given nembutal at 60 mgm./kgm. intraperitoneally, the mice were exposed. The results are given in Table 1.

TABLE 1

SURVIVAL FOLLOWING VARIOUS DOSES OF IRRADIATION TO MICE UNDER NEMBUTAL AND WITH SPLEENS SHIELDED EXTERNALLY

X-ray Dose in r	Survival Rates (30 Days)	Mean Death Time (Days)	% Survival (30 Day)
800	8/10	....	80
900	13/29	11.7	45
1000	3/20	6.5	15
1100	1/10	6	10
1200	0/20	7	0

If the data in the above table are plotted on log-probability paper, it can be seen that the LD_{50} is about 890 r, the coefficient of variation being about 14%.

Four groups, each containing ten animals, were given 350, 400, 450, and 500 r to check the previous LD_{50} for normal male mice. The respective 30-day survivals of 8/10, 6/10, 2/10, and 3/10 give an estimated LD_{50} of about 420 r as compared with the more stable value of 450 r obtained previously (1). Since these values are essentially the same, we may compare LD_{50} values directly with those given previously. Using an LD_{50} value of 420 r we obtain a dose reduction factor of 2.1 for spleen shielding in the above situation.

To determine the effectiveness of the shielding, experiments were carried out using a Victoreen with the tip shielded by the lead wedges, the lead being held on by a piece of tape. The following results were obtained. If the Victoreen was suspended in air, then the reading was about 4% of that in the absence of the lead shield. If the Victoreen was set on the lucite plate (1") which is used for exposing mice in the screening program, the reading was from 8 to 15% of that without lead, depending on the placement. If the Victoreen was placed on the body of a dead mouse, with a mouse on either side and near the top, then a reading as high as 20% of the unshielded value could be obtained. Much of the spleen is closer to the lead shield than the center of the ionization chamber so that at least part of the spleen may be exposed to less than 200 r for a 1,000 r exposure. But the outer edges of the spleen undoubtedly are exposed to substantially more than 200 r. However, it is likely that the central portion, which is most protected, is the only part that is important for the survival of sufficient cells to serve as a source for repopulating the reticulo-endothelial system. In any case, it must be kept in mind that the shielding is by no means complete. Further studies are needed to find out whether or not complete shielding of the exteriorized spleen, or the injection of young spleen cells or bone marrow increases protection above that obtained with the external shielding.

Protection against irradiation by an external shield over the spleen together with MEA or serotonin. A dose of 400 mgm./kgm. of mercaptoethylamine (MEA) 15 minutes prior to irradiation affords about the same protection as does an external shield over the spleen. It is of interest to find out the extent of this protection if the spleen is simultaneously shielded externally. The additional protection could come from further protection of the spleen or from protection against other systems. Since the doses of radiation are so high that death by gut damage is beginning (2), and since MEA protects against this damage (2,3), a synergistic action is not unexpected.

Intraperitoneal administration of serotonin (5-hydroxytryptamine) at levels above 50 mgm./kgm. results in a substantial protection against subsequent irradiation. A dose reduction factor of about 1.8 has been found (4). Simultaneous protection by serotonin and shielding could result in some additional protection of the spleen, but also in a slight decrease in damage to the gastrointestinal tract. Experiments were, therefore, carried out from which an estimate could be made of the extent of the interaction.

In Table 2 are given data on survival of irradiated mice which were shielded over the spleen and given either MEA or serotonin.

Protection against irradiation by repeated administration of hydroxylamine. Hydroxylamine produces toxic symptoms quickly in male CF_1 mice but considerable amounts can be administered when given over a period of time and especially if the mice are previously given nembutal. An attempt was made to increase the protection against irradiation by giving repeated injections of 50 mgm./kgm. at 5-minute intervals beginning 25 minutes after administration of 70 mgm./kgm. of nembutal. Five minutes after the fourth injection of hydroxylamine the mice were given various doses of irradiation at a rate of 600 r per minute. In preliminary experiments, after survival seemed better in those animals given methylene blue (20 mgm./kgm. intraperitoneally) after irradiation and hence this procedure was finally adopted. A few animals in

TABLE 2

SURVIVAL OF MALE MICE WITH EXTERNAL SHIELD OVER THE SPLEEN AND WITH MEA (400 MGM./KGM.) OR SEROTONIN (100 MGM./KGM.)

X-ray Dose in r	Pre-X-ray Treatment	Survival Ratio (30-Day)	Mean Death Time in Days	% Survival (30-Day
1,000	Serotonin (I.P.) 100 mgm./kgm.	8/10	....	80
1,100	"	8/18	8.8	44.5
1,200	"	1/8	10	12
800	MEA (I.P.) 400 mgm./kgm.	9/10	....	90
1,000	"	15/19	11	79
1,200	"	12/20	13	60
1,300	"	13/19	10.4	68
1,400	"	8/20	11.1	40

each group of ten can be expected to die just before, during, or after irradiation. Animals surviving for a few minutes after being given methylene blue lived several days or more. In Table 3 only such animals are included.

TABLE 3

PROTECTION AGAINST IRRADIATION BY INTRAPERITONEAL INJECTION OF HYDROXYLAMINE HYDROCHLORIDE IN MALE MICE

X-ray Dose in r	Pre-X-ray Treatment with Hydroxylamine Hydrochloride	Survival Ratio	Mean Death Time in Days	% Survival (30-Day)
0	200 mgm./kgm. (4 doses)	10/10	....	100
800	50 mgm./kgm. (single dose)	1/10	7.5	10
800	200 mgm./kgm. (4 doses)	13/23	13.1	57
1,000	200 mgm./kgm. (4 doses)	1/12	11	8

At 50 mgm./kgm. there were no drug deaths. The first death of the irradiated mice occurred on the sixth day. No pretreatment with nembutal nor posttreatment with methylene blue was used in this case. The data suggest only a slight protection against 800 r by 50 mgm./kgm. of hydroxylamine when given intraperitoneally seven minutes before irradiation. Pretreatment with nembutal alone (85 mgm./kgm.) results in only a low percentage survival at 600 r (see below).

Since over one-half of the mice survived after 200 mgm./kgm. of NH_2OH (hydroxylamine) the dose reduction factor for this situation is not far from 2. Hence hydroxylamine can give considerable protection against ionizing irradiation when given in sufficiently large amounts before irradiation.

Protection against irradiation by very low oxygen tension. The environmental oxygen tension of male mice was carefully brought to a level of about 4.6% oxygen and the animals exposed to various levels of irradiation given in two minutes or less.

After the mice had been placed in the plastic exposure chamber for a few minutes, the oxygen tension was dropped to about 10% for one minute. The tension was then dropped to about 7% for a half minute and then to 4.6% during

a half minute interval. Irradiation was begun 20 seconds after the tension reached 4.6%. At the moment the x-ray machine shut off, a solenoid allowed air to replace the low oxygen mixture in the exposure chamber. Few animals died during the 1-1/2 minute exposures (900 r). However, for exposures of two minutes (1,200 r), the anoxia results in early 50% mortality. In Table 4 only survivors of the exposure period are included.

TABLE 4

SURVIVAL OF MALE MICE FOLLOWING X-IRRADIATION DURING 4.6% OXYGEN TENSION

X-ray Dose in r	Survival Ratio (30-Day)	Mean Death Time (Days)	% Survival (30-Day)
800	11/14	(11)	79
900	8/11	(11)	73
1,000	11/20	13.3	55
1,200	1/16	10.7	6

From the above table it can be seen that the LD_{50} (30-day) is somewhat over 1,000 r, giving a dose reduction factor between 2 and 2-1/2 for this situation.

An additional group of ten mice was given 550 r. As the x-ray machine shut off, the air in the chamber was rapidly replaced with nitrogen. Ten seconds later the level was raised to 5% O_2 and 10 seconds later to 7%. Since the abrupt drop produces excitement, it was not possible to maintain the oxygen level as low as 5% even for a minute. After two minutes the mice were removed from the exposure chamber. No animal survived beyond 11 days, the mean time of death being 9.3 days. Untreated animals given 550 r would give no survivors in most instances. Hence, production of anoxia within 10 seconds after irradiation failed to show protection from the rather low dose of 550 r.

A few trials were made to combine anoxia with 600 mgm./kgm. of MEA (I.P.) with the x-ray exposure being given 15 minutes after injection of MEA. Since a higher dose of irradiation needed to be given and since there seems to be some increased sensitivity to anoxia in the nembutalized-MEA treated animals, the level of anoxia could not be maintained below 6%. In a group of eight mice given 60 mgm./kgm. of nembutal at 30 minutes, 600 mgm./kgm. of MEA at 15 minutes, and 6% O_2 at zero time and during irradiation (1,500 r), there were no survivors after 14 days, the mean death time being 10 days. Another group of seven mice was exposed to 1,600 r. Two of these animals survived 30 days. The mean death time of those failing to survive 30 days was 13 days.

Protection against irradiation by sodium cyanide in nembutalized male mice. Previous studies (5) showed that the oxygen level in most mice under nembutal given 4 mgm./kgm. of sodium cyanide intraperitoneally begins to fall at about three minutes. Only 1 in 6 showed a decrease at the end of the first minute. Most of the others showed an appreciable increase during the first two minutes. In terms of protection due to oxygen deficit one would not expect protection to be exhibited until about three minutes or more following injection of sodium cyanide. Hence, nembutalized mice were given 700 r after 1, 3, 8, and 16 minutes after sodium cyanide and the results are shown in Table 5.

TABLE 5

SURVIVAL IN NEMBUTALIZED MALE MICE FOLLOWING IRRADIATION AND PRETREATMENT WITH SODIUM CYANIDE

X-ray Dose in r	Time After NaCN	Survival Ratio (30-Day)	Mean Death Time (Days)	% Survival (30 Days)
700	No NaCN	0/16	9.5	0
700	1'	3/15	11.6	20
700	3.5'	13/34	11.7	38
700	8'	5/24	13.7	21
700	16'	1/18	9.7	5

From the above results it can be seen that when the irradiation is given at about 1.7 minutes mean time from injection of NaCN, there is appreciable protection. The survival is greater at four minutes mean time but the increased survival is not significant, though significantly greater than the control. By 16 minutes the survival is negligible. The data do suggest, however, that protection develops too rapidly to be accounted for by the change in oxygen tension. The data given here should be compared with those of Dilley and Doull (6) in which nembutal was not given. They found considerable protection by potassium cyanide against 700 r when the animals were irradiated one minute after injection.

Protection against ionising irradiation by topical application of histamine to the spleen of male CF$_1$ mice. As part of a program to study the extent of protection by topical application of small amounts of drugs to the spleen before irradiation, histamine dichlorate has been used. The spleens were exposed and a small piece of Saranwrap placed underneath to form a small trough. The animals were carefully placed in the exposure chamber. The drug was applied drop by drop over a period of about two minutes and the liquid in the plastic trough was stirred and used to bathe the spleen until time to place the exposure chamber under the x-ray beam.

In Table 6 are also given data in which the drug was administered intraperitoneally and intravenously using a volume equal to 1% body weight. In one experiment the drug was injected directly into three parts of the spleen using a volume of 1/4% body weight. The results are shown in the following table.

TABLE 6

SURVIVAL IN MALE MICE IRRADIATED FOLLOWING TOPICAL APPLICATION OF HISTAMINE DICHLORIDE TO THE SPLEEN

X-ray Dose in r	Treatment (mgm./kgm., route, and time at x-ray)	Survival Ratio (30-Day)	Mean Death Time	% Survival (30-Day)
700	None	0/30	8.5	0
700	250, I.P., 5'	1/10	8.5	10
700	250 (neut.), I.V., 5'	4/10	12	40
700	250, spleen inj., 5'	4/8	21	50
700	250, topical, 5'	2/10	9	20
800	250, I.P.	0/8	10.5	0
800	250 (neut.), 5'	6/9	(13)	67
800	250, topical, 3'	1/14	11.2	7
800	250, I.P.	0/10	9.9	0
800	300, I.P.	0/10	8.6	0
800	500, I.P.	0/10	9.4	0

From the above it can be seen that in these mice intraperitoneal injection of histamine is not very effective. In all these cases, the injections were made into the abdominal cavity away from the spleen. On the other hand, these preliminary results suggest that intravenous injection of neutralized histamine dichloride, topical application, and injection into the spleen result in substantial protection. When the injection is made into the spleen, the site of injection shows a marked blanching. Topical application results in a blotching and darkening appearance.

In the earlier experiments (700 r), the drug was applied to the capsule only until near the end of the period. A few attempts to recover the unabsorbed drug indicate that the capsule absorbs very little in time while that which spills over the capsule so as to wet the sides of the spleen and the attached tissue is absorbed rather quickly.

It may be noted that the neutralized solutions seem more effective. The possibility needs to be considered that the irritating effect of the low pH may partially counteract the constricting effect of the drug. It should be pointed out also that the role of timing needs further study.

Protection against ionising irradiation by topical application of aminoethylisothiouronium dichloride (AET) or by electrical shock to spleens of male mice. The effect of topical application of AET to exposed spleens of nembutalised mice has been studied. The drug was applied as described above, not neutralised unless otherwise indicated. Included in this series was a group in which the spleens were shocked by a very short monophasic pulse. Three shocks were delivered to different parts of the exposed spleen. Thirty seconds elapsed until irradiation (600 r per minute) was begun. The intensity of each shock was sufficient to produce a strong twitch when applied to muscle. No movement except that due to current spread was noted. Relatively little change in the appearance of the spleen was seen. A few animals in which the spleen oxygen tension was measured showed small to moderate decreases, with some increasing slightly. Although this result would not suggest that spleen shock should give protection through spleen anoxia, some experiments were carried out nevertheless. The results of these experiments are shown in Table 7.

TABLE 7

THE EFFECT OF TOPICAL APPLICATION OF AET OR SPLEEN SHOCK ON SURVIVAL OF MALE MICE AFTER X-IRRADIATION

X-ray Dose in r	Treatment	Dose of AET	Survival Ratio (30-Day)	Mean Death Time (Day)	% Survival (30-Day)
600	None	0	2/10	10.4	20
600	Spleen exposed	0	1/10	11.2	10
600	Spleen shock	0	3/10	9	30
700	Spleen shock	0	3/28	9.2	11
700	Spleen exposed	0	0/20	10.4	0
800	Topical	75	1/10	13	10
800	Topical	100	0/10	8.6	0
800	Topical	125	2/10	17	20
800	Topical	125 (neut.)	4/10	14	40

From the data given above, it can be seen that there is little or no protection due to spleen exposure or spleen shock, over and above that due to the nembutal (85 mgm./kgm.) administered about one-half hour before irradiation. On the other hand, topical application of AET at 125 mgm./kgm. at 1/4% body weight, especially a neutralised solution, appears to give appreciable protection. Whether or not this protection exceeds that for an intravenous injection cannot be determined without additional data.

Protection against ionizing radiation following administration of MEA and $NaNO_2$. Since 400 mgm./kgm. of MEA given 15 minutes before irradiation and 200 mgm./kgm. of $NaNO_2$ 20 minutes before irradiation give roughly the same protection but neither produced appreciable survival at 1,300 r, an attempt was made to test the effect of giving both. In the absence of interaction between the two compounds such that either reduces the effect of the other, then in view of the synergistic effect of anoxia or spleen shielding and MEA, one might expect at least the anoxic effect of $NaNO_2$ to act synergistically with MEA. The procedure used to test the hypothesis was as follows. At time zero one mouse was given 200 mgm./kgm. $NaNO_2$ intraperitoneally. At 5 minutes 400 mgm./kgm. MEA was administered. At 20 minutes irradiation was begun. It lasted 2-1/6 minutes. As quickly as possible 20 mgm./kgm. of methylene blue was administered intravenously to prevent death from the nitrite. The animal was then given 75 mgm./kgm. nembutal to reduce mortality from the MEA convulsions.

The result from 15 animals surviving five minutes past administration of nembutal was as follows. Of 13 surviving overnight, six survived 1,300 r for 30 days. No survivors would be expected from either agent alone, indicating an additive effect.

Summary

1. Use of an external 1/4 inch lead shield (0.1 x 2 x 0.7 cm.) placed over the spleen of mice increased the LD_{50} for whole-body x-ray exposure from about 450 r to 890 r. The administration of serotonin (100 mgm./kgm. intraperitoneally) in addition to the external shielding of the spleen increased the LD_{50} to about 1,075 r whereas MEA (400 mgm./kgm. intraperitoneally) plus spleen shielding increased the LD_{50} to about 1,200 r. MEA alone was about as effective as spleen shielding alone.

2. Hydroxylamine given prior to whole-body x-ray exposure at a dosage level of 50 mgm./kgm. results in a slight protection but increasing the dose to 200 mgm./kgm. (given over a period of 15 minutes to minimize toxicity) increases the LD_{50} of whole-body x-ray exposure to over 800 r giving a dose reduction factor (DRF) of close to 2.

3. Anoxia produced by exposing mice to an environmental oxygen concentration of about 4.6% increased the LD_{50} of whole-body x-ray exposure to about 1,000 r. Production of anoxia within 10 seconds after x-ray exposure gave no protective effect.

4. Administration of sodium cyanide at different times prior to an x-ray exposure of 700 r showed that the greatest survival occurred when the agent was given at four minutes prior to the irradiation.

5. Experiments involving the topical application of drugs to the spleen gave the following results: substantial degrees of protection could be obtained with histamine and 2-aminoethylisothiourea but the amounts required to produce protection were comparable to the amount of each agent required to produce radioprotective effects when given intravenously. Electric shocks to the spleen failed to yield a significant protective effect.

6. Administration of 400 mgm./kgm. of MEA and 200 mgm./kgm. of $NaNO_2$ before 1,300 r resulted in 30-day survivors indicating an additive protective effect.

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THE INFLUENCE OF EXPOSURE TO LOW LEVELS OF GAMMA OR FAST NEUTRON IRRADIATION ON THE LIFE SPAN OF ANIMALS

I. Early and Late Radioprotective Effects in Proton-Irradiated Mice Pretreated with Chemical Protectors

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This report concerns: Radioprotective effects in mice total-body irradiated by high energy (140 Mev) protons following pre-irradiation treatment with 2-mercaptoethylamine hydrochloride (MEA) and with p-aminopropiophenone (PAPP), and a comparison with similar effects in mice irradiated by 250 Kv x-rays.

Immediate or ultimate application of the results: This investigation has relevance to at least three significant radioprotection problems. These are, the extent to which a selected agent can modify the response of a biological system to one particular type of radiation--specifically, high-energy protons; the relative effectiveness of two different types of radioprotective agents in modifying the response of a biological system to this type of radiation; and the relative effectiveness of one type of radioprotective agent compared in systems exposed to two different types of radiations--protons and x-rays. The methods and results of a first series of experiments (Series A) designed to investigate these problems has been given in a preceding report (1). The present report contains data obtained in a second series of experiments (Series B) replicating and extending the range of radiation doses employed in the first series. Initial analysis of the data of both series of experiments confirms the original conclusion that pre-irradiation treatment with MEA or PAPP is able to reduce 30-day lethality in mice exposed to high-energy protons. In addition, examination of the series A survival data out to 140 days has both indicated the need for, and suggested an approach to, a more sensitive measure of radiation response than the conventional indices provide. Experimental and theoretical bases have thus been provided for further studies of radioprotection by MEA and PAPP at the present and also at lower proton energies.

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The experiments of Series A employed a range of radiation doses which, for protected animals, produced mortality only approaching the 50% point. Hence, mid-lethal doses, $\bar{D}$, had to be obtained by extrapolation. The various effectiveness indices (dose reduction factor, relative biological effectiveness, relative protective effectiveness) were, therefore, each afflicted with an uncertainty related to that of the two $\bar{D}$'s from which they were calculated. The purpose of the Series B experiments was to provide a firmer estimate of the various mid-lethal doses by using larger radiation doses for the protected groups, as well as doses overlapping some of the Series A points.

An additional question raised by the Series A experiments was whether the slopes of the mortality versus dose lines were unduly low due to the randomization over several age groups (14 to 20 weeks) which the experimental plan required. To obtain information on this point, regression lines for protected and unprotected mice homogeneous in age to within one week were obtained in the Series B experiments. These regression lines could then be compared with similar lines obtained using mice having a larger spread in age.

Physical methods and materials. The proton beam used for the Series B experiments was the same as that used for Series A. This beam can be specified by the following parameters:

Accelerator: 170-inch synchrocyclotron
Energy: 440 Mev at extraction
Pulse width: 400 microseconds
Pulse repetition rate: 70 pulses per second.

A more detailed description of the beam and irradiation geometry used has been given previously (1). The average dose rate on the midline of a cylindrical water phantom on the vertically scanned irradiation plate was maximally about 40 rad/min. for the Series B runs. This is approximately half the rate obtained in the Series A runs. The reason for this difference is not definitely known. One possibility is that either the particle trajectory or the efficiency of the extraction apparatus was altered due to modifications made after Series A in iron structures adjacent to the machine. The dose distribution over the surface of the irradiation plate, however, was the same for the two runs, as judged both from film exposures and from ion chamber readings on the plate.

Specifications of the x-ray beam used for the Series B runs were as follows:

Generator: Keleket
Energy: 250 Kvp
Tube current: 15 ma.
External filter: 1/4 mm. copper plus 1 mm. aluminum
FSD: 75 cm.
Backscatter material: lucite
External collimator: none
Beam diameter: approximately 45 cm.

Pending a more detailed comparison of the generator used during Series B with that used during Series A, the same roentgen-to-rad conversion factor adopted previously (1) will be assumed to hold for Series B.

The final item of dosimetry concerns the charge-calibration of the Victoreen chamber. Immediately preceding the Series B runs, the Victoreen set (chamber and charger) was recalibrated against the Argonne Cancer Research Hospital (ACRH) cobalt-60 source; this calibration was then compared with a commercial cobalt-60 calibration (Victoreen Instrument Company) of the same set for similar geometric conditions performed six days before. The conditions and results of these calibrations are shown in Table 1. Since the commerical calibration is accurate to only a few per cent, the agreement is quite

satisfactory. Thus it was felt that the charge-calibration for Series B had been somewhat more reliably established than that for Series A. Quite apart from this, a new value for the half-life of cobalt-60 was recently published (2). This circumstance, along with the relative status of the calibrations, has led us to choose the exposure dose rate used for the Series B calibration (45.1 r/minute on 2/15/63) as the reference rate. The value for the Series A calibration was then corrected to this rate taking for the half-life of cobalt-60 the new value of 5.263 years (1922.3 days). The correction required for the Series A doses turned out to be negligible (less than 1%). However, this calibration, and the more accurate value for the half-life of cobalt-60, will aid in referencing the Victoreen set using the radium calibration source now available in this laboratory.

TABLE 1

COBALT-60 CALIBRATION CONDITIONS AND RESULTS

	Source to Chamber Distance	Field Size	Correction Factor (for 22° C.)
ACRH	81.6 cm.	10 cm. x 10 cm. (square)	1.025
Victoreen	50.0 cm.	10 cm. diameter (circular)	1.037
			1.031 ± 0.006 av. (0.6%)

Biological materials and methods. Mice used in this experiment were Carworth Farms CF_1, caged not more than 12 per stainless steel cage (7" H, 9" W, 13" L) in which food (Rockland Mouse Diet) and drinking water were available ad libitum. All cages were housed in a single room and were inspected for dead mice daily and cleaned weekly. Both proton and x-ray irradiations were carried on during successive 8 to 12-hour periods spanning several days. The mice used in any particular period were randomized just prior to the beginning of that period within the age groups 19 ± 1, 21 ± 1, and 23 ± 1 weeks for male mice, and 18 to 19 weeks for female mice. An additional group of male mice 14 to 15 weeks of age was used in the x-ray irradiations.

Chemical agents used in this study were made up in concentrations that permitted the use of an injection volume corresponding to less than 2% of the body weight of the mouse. PAPP was prepared by dissolving C.P. grade reagent in propylene glycol with gentle heating and then diluting with an equal volume of triple-distilled water. The concentration of the final solution was 3 mgm./milliliter. MEA was prepared by dissolving the C.P. grade reagent in triple-distilled water. The concentration of the final solution was 22.5 mgm./ml. Half of the control mice received one, and half of the control mice the other, of the above diluents.

In the proton irradiations, mice were processed in batches of nine; all nine received the same drug dose and the same radiation dose. A sample of each of the age groups was used for each exposure at each radiation dose level.

Injections were given intraperitoneally using a 1 ml. syringe and 22 gauge needle, 1/2 inch long. After injection, mice were placed in vented centrifuge tubes. A delay of five minutes was interposed between the end of the injections and the start of irradiation, during which time the tubes were attached to the lucite plate. The plate containing tubes and mice moved vertically up and down past the fixed beam continuously during irradiation. After delivering a measured radiation dose to this batch of mice, the tubes were removed, and the mice sorted into appropriate cages. The propylene glycol and the water control mice were caged together, but were permanently marked so as to be distinguishable. The female control mice were given water only.

The x-ray irradiations commenced one week after the end of the proton irradiations. The biological procedures used for irradiations were exactly comparable with those used for proton irradiations, except that the mice were processed in batches of sixteen, and the lucite disc carrying the mice rotated continuously within the beam about a vertical axis.

Results

The cumulative per cent mortality, p, is shown in Table 2 for both Series A and B runs. For protons, the data has been cumulated to 30 days for A and 28 days for B; for x-rays, the data has been cumulated to 30 days for A and to 21 days for B. Data beyond 21 and 28 days is not yet available for the B series experiments. However, data presented below and discussed more fully later in the report indicate that early mortality is relatively fully expressed by 21 days.

The standard deviation, σ , is calculated on the assumption that deaths within each group of mice at a particular dose level obey binomial statistics, and that the per cent mortality observed in this sample is a reasonable estimate of the per cent mortality and would be observed in a large population of mice. The number n is the original number of mice irradiated, not including any that died during irradiation or during the first two days post-irradiation. In the control groups, the number of mice excluded from analysis due to immediate death (during irradiation) or due to short-term death (two days post-irradiation) is negligible. In groups receiving MEA or PAPP before irradiation, the excluded mice comprise about 5 to 15% of the initial number. The three age groups irradiated during the same or successive treatment periods all exhibited essentially the same survival behavior and the groups were pooled for analysis.

The data in Table 2 are plotted in Figures 1 through 10, inclusive, in probit units of mortality versus dose. The straight-line fits to these data have been estimated by eye. The 21-day x-ray data of Series B appears to agree reasonably well with the 30-day x-ray data of Series A. The same may be said of the Series A and B proton data.

TABLE 2

MORTALITY DATA FOR CF_1 MICE EXPOSED TO VARIOUS DOSES OF PROTON X-RADIATION

Series A:	Males 14 to 20 Weeks Old									Females 14 to 16 Weeks Old		
Dose (rads)	Control			PAPP			MEA			Control		
	n	p%	± σ	n	p%	± σ	n	p%	± σ	n	p%	± σ
440 Mev protons: 30 days post-irradiation												
235	35	0	1.4	36	0	1.4	41	2.4	2.4	..	....	....
351	36	13.9	5.8	37	2.7	2.6	37	0	1.4	18	0	2.8
468	36	8.3	4.6	35	0	1.4	37	0	1.4	18	5.6	5.4
591	36	25.0	7.2	35	14.3	5.9	35	11.4	5.4	18	50.0	11.8
702	35	51.4	8.4	34	17.6	6.5	37	13.5	5.6	18	44.4	11.7
820	36	55.6	8.3	34	14.7	6.1	35	17.1	6.4	..	....	....
939	35	71.4	7.6	36	47.2	8.3	34	23.5	7.1	..	....	....
250 Kvp x-rays; 30 days post-irradiation												
222	36	2.7	2.7	..	....	...	..	....	...	..	....	....
333	35	14.3	5.9	..	....	...	..	....	...	18	5.5	5.4
444	36	27.7	7.5	38	13.2	5.5	41	2.4	2.4	18	11.0	7.4
555	36	66.7	7.9	40	12.5	5.2	40	5.0	3.5	18	44.4	11.7
666	36	66.7	7.9	36	25.0	7.2	38	28.9	7.3	18	83.3	8.8
777	..	....	...	35	31.4	7.8	41	39.0	7.6	..	....	....
888	..	....	...	36	27.8	7.5	41 -	51.2	7.8	..	....	....

TABLE 2--Continued

Series B: Males 18 to 24 Weeks Old

Control				PAPP				MEA			
Dose (rads)	n	p%	± σ	Dose (rads)	n	p%	± σ	Dose (rads)	n	p%	± σ
440 Mev protons; 28 days post-irradiation											
390	36	2.8	2.8	873	30	26.7	8.1	971	33	18.2	6.7
487	36	19.4	6.5	1116	35	37.1	8.2	1255	29	41.4	9.2
618	35	28.6	7.7	1347	34	44.1	8.5	1395	24	50.0	10.2
716	36	41.7	8.7	....	..	.....	...	....	..	.....	....
824	35	51.4	7.7	....	..	.....	...	....	..	.....	....
935	36	47.2	8.3	....	..	.....	...	....	..	.....	....
1037	36	63.9	8.0	....	..	.....	...	....	..	.....	....
250 Kvp x-rays; 21 days post-irradiation											
402	32	9.4	5.1	629	32	25.0	7.7	651	32	18.7	6.9
532	32	28.1	7.9	770	25	44.0	9.9	749	31	58.1	8.9
608	32	62.5	8.5	900	31	54.8	9.0	814	32	40.6	8.7
683	32	75.0	7.7	1063	32	50.0	8.9	900	32	78.1	7.3
765	32	78.1	7.3	1215	31	96.8	3.1	982	32	100.0	1.6
841	32	96.9	3.0	1346	31	100.0	1.6	1074	32	100.0	1.6
949	32	100.0	1.6	1465	30	100.0	1.7	1156	30	70.0	8.4
				1628	29	100.0	1.7				
				1844	30	100.0	1.7				

TABLE 2--Continued

Series B: Males 14 to 15 Weeks Old								Series B: Control Females 18 to 19 Weeks Old							
Control				MEA											
Dose (rads)	n	p%	±σ	Dose (rads)	n	p%	±σ	Dose (rads)	n	p%	±σ	Dose (rads)	n	p%	±σ
250 Kvp x-rays; 21 days post-irradiation								440 Mev protons; 28 days post-irradiation				250 Kvp x-rays; 21 days post-irradiation			
445	16	12.5	8.4	667	32	21.9	7.3	509	18	27.8	10.6	463	16	50.0	13
521	16	31.2	11.5	781	32	25.0	7.7	606	18	38.9	11.5	543	16	62.5	12
575	16	93.7	5.9	862	29	69.0	8.6	670	18	33.3	11.1	599	16	50.0	13
629	16	56.2	12.4	944	32	68.7	8.2	757	18	66.7	11.1	656	16	62.5	12
683	16	81.2	9.8	1025	32	78.1	7.3					712	16	100.0	3
738	16	68.7	11.6	1107	32	81.2	6.9					833	16	100.0	3
814	16	100.0	3.0	1221	29	96.6	3.2					892	16	100.0	3
846	16	100.0	3.0												

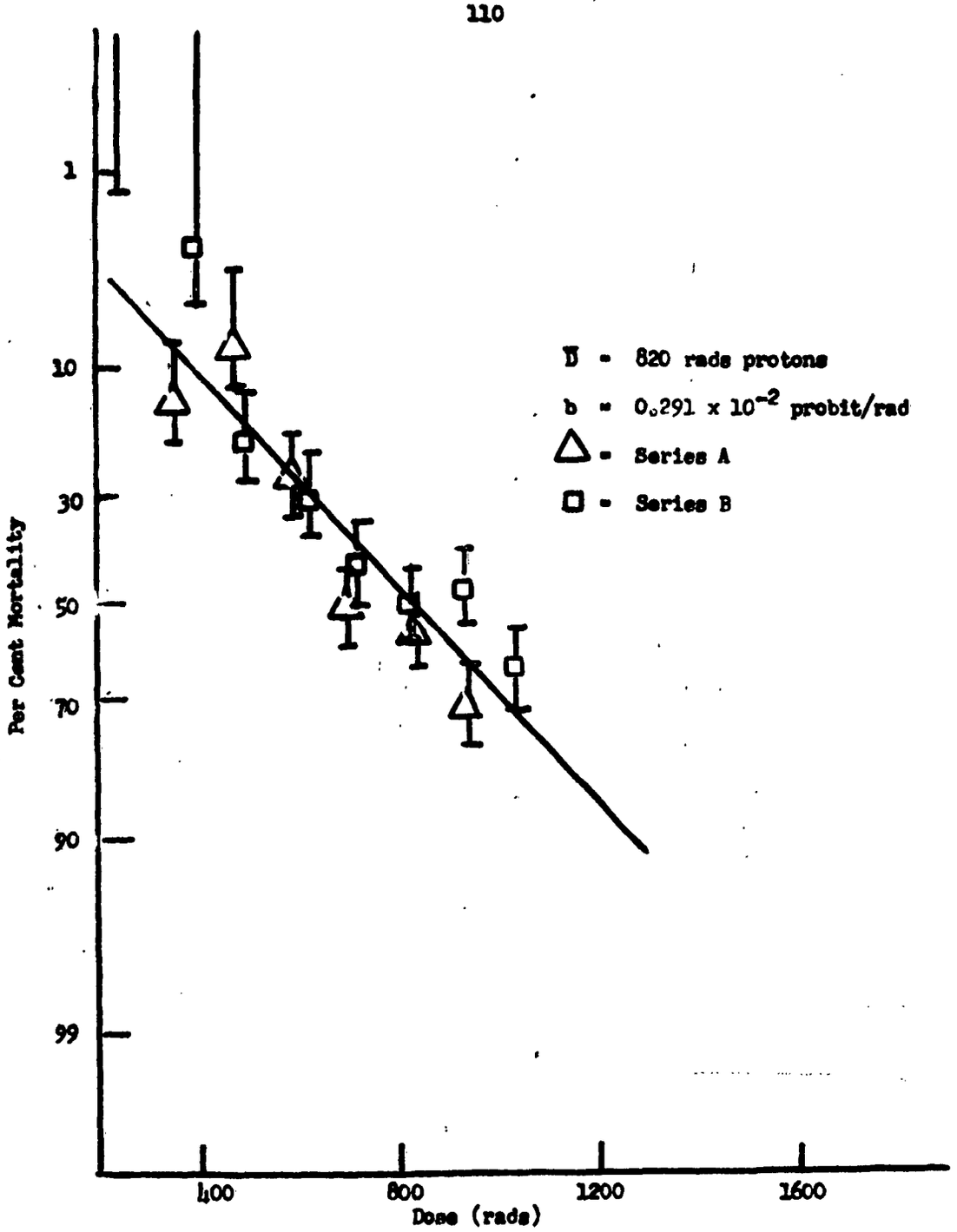

Figure 1. Dose-mortality data for 19 ± 5 weeks old CF_1 male mice given vehicle only five minutes prior to whole-body proton irradiation.

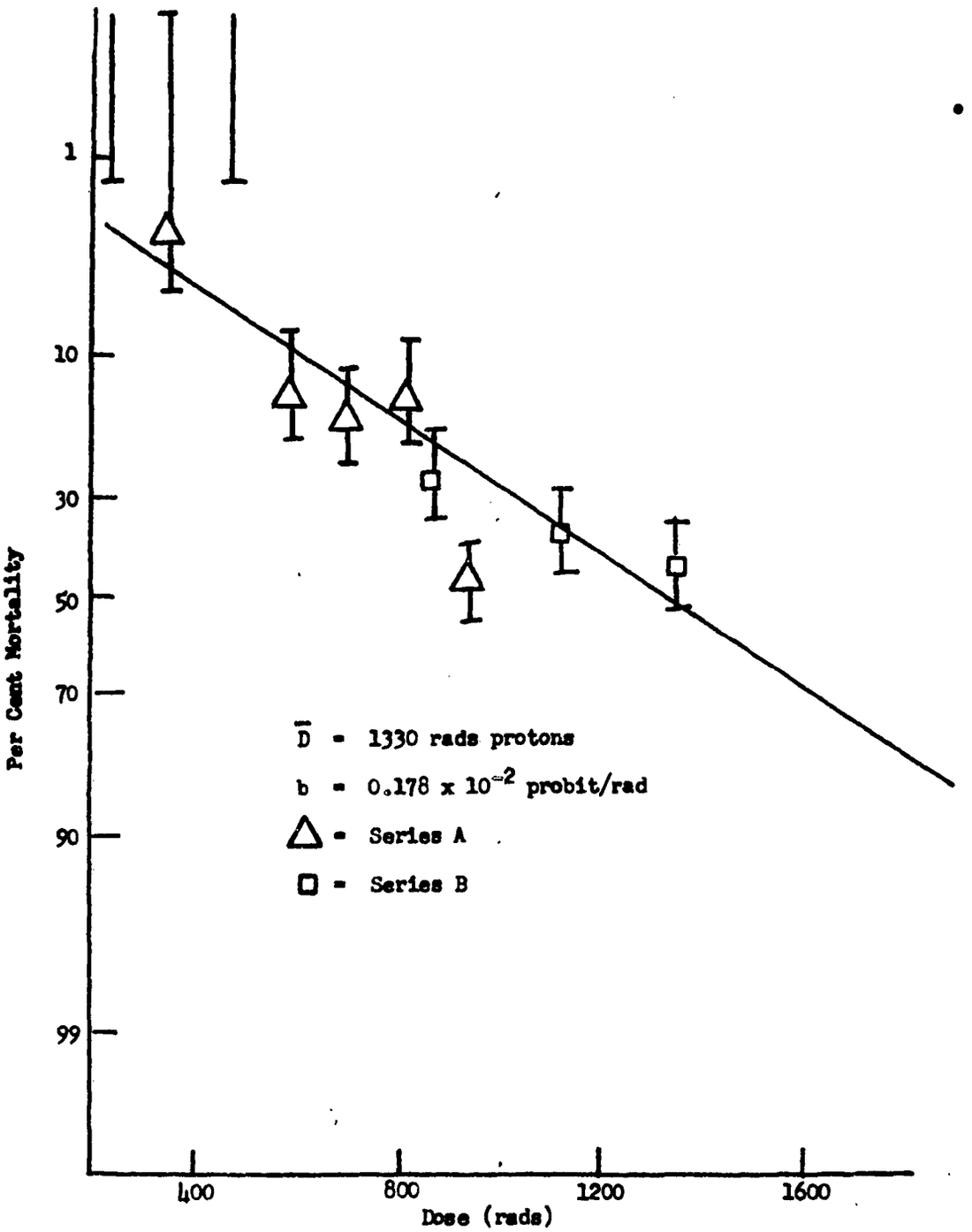

Figure 2. Dose-mortality data for 19 ± 5 weeks old CF_1 male mice given 30 mgm./kgm. PAPP five minutes prior to whole-body proton irradiation.

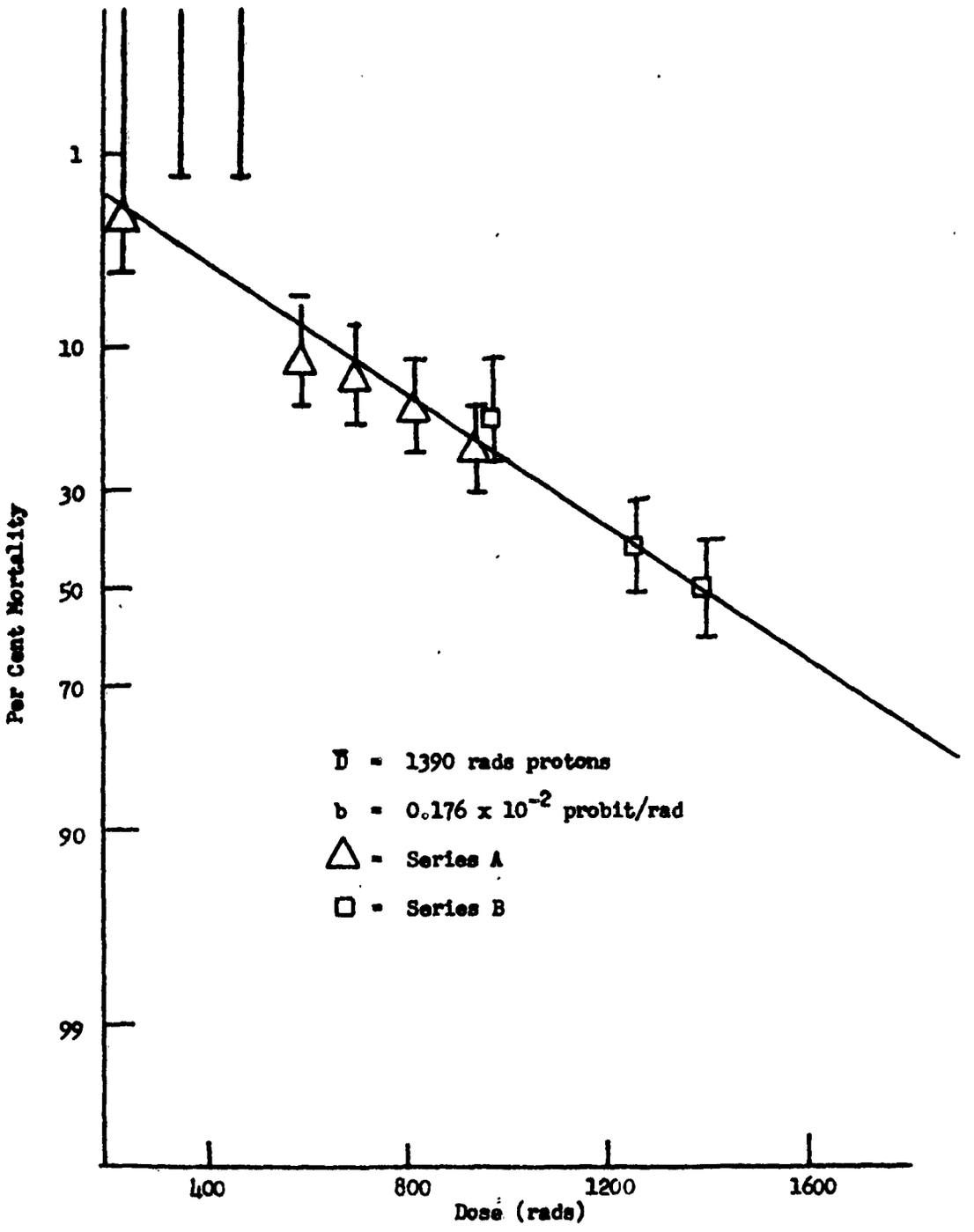

Figure 3. Dose-mortality data for 19 ± 5 weeks old CF_1 male mice given 225 mgm./kgm. MEA five minutes prior to whole-body proton irradiation.

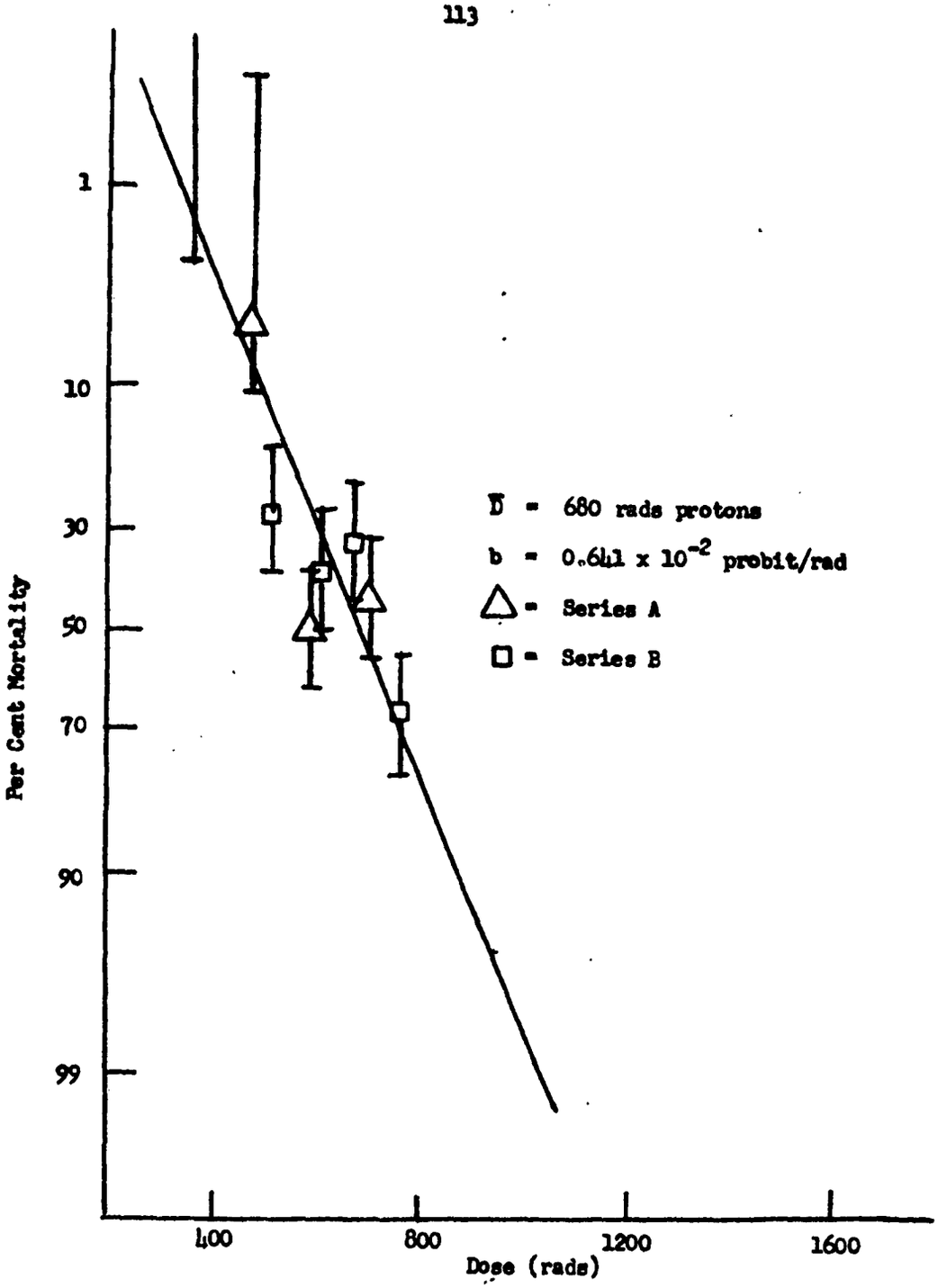

Figure 4. Dose-mortality data for 18 to 19 weeks old CF_1 female mice given vehicle only five minutes prior to whole-body proton irradiation.

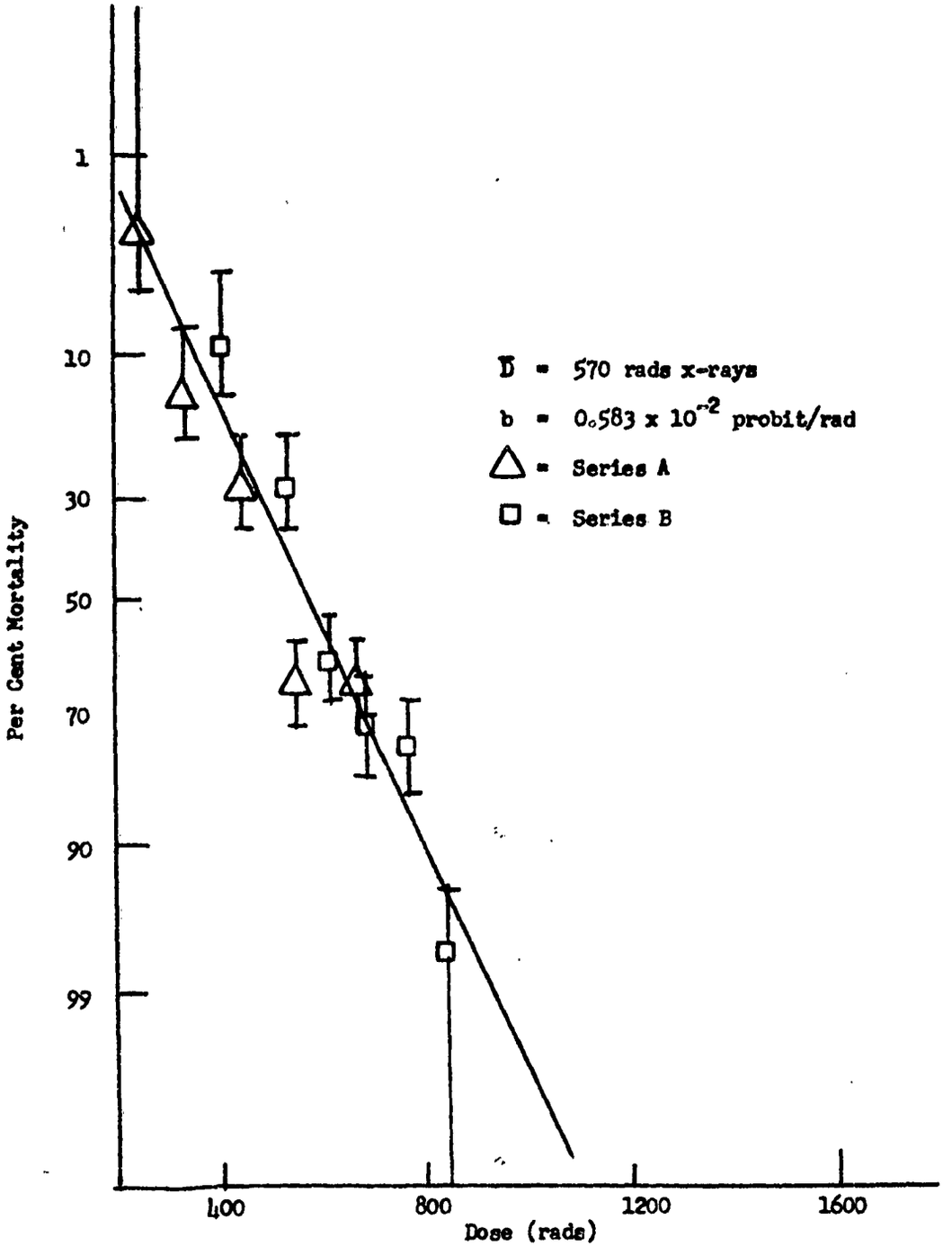

Figure 5. Dose-mortality data for 19 ± 5 weeks old CF_1 male mice given vehicle only five minutes prior to whole-body x-irradiation.

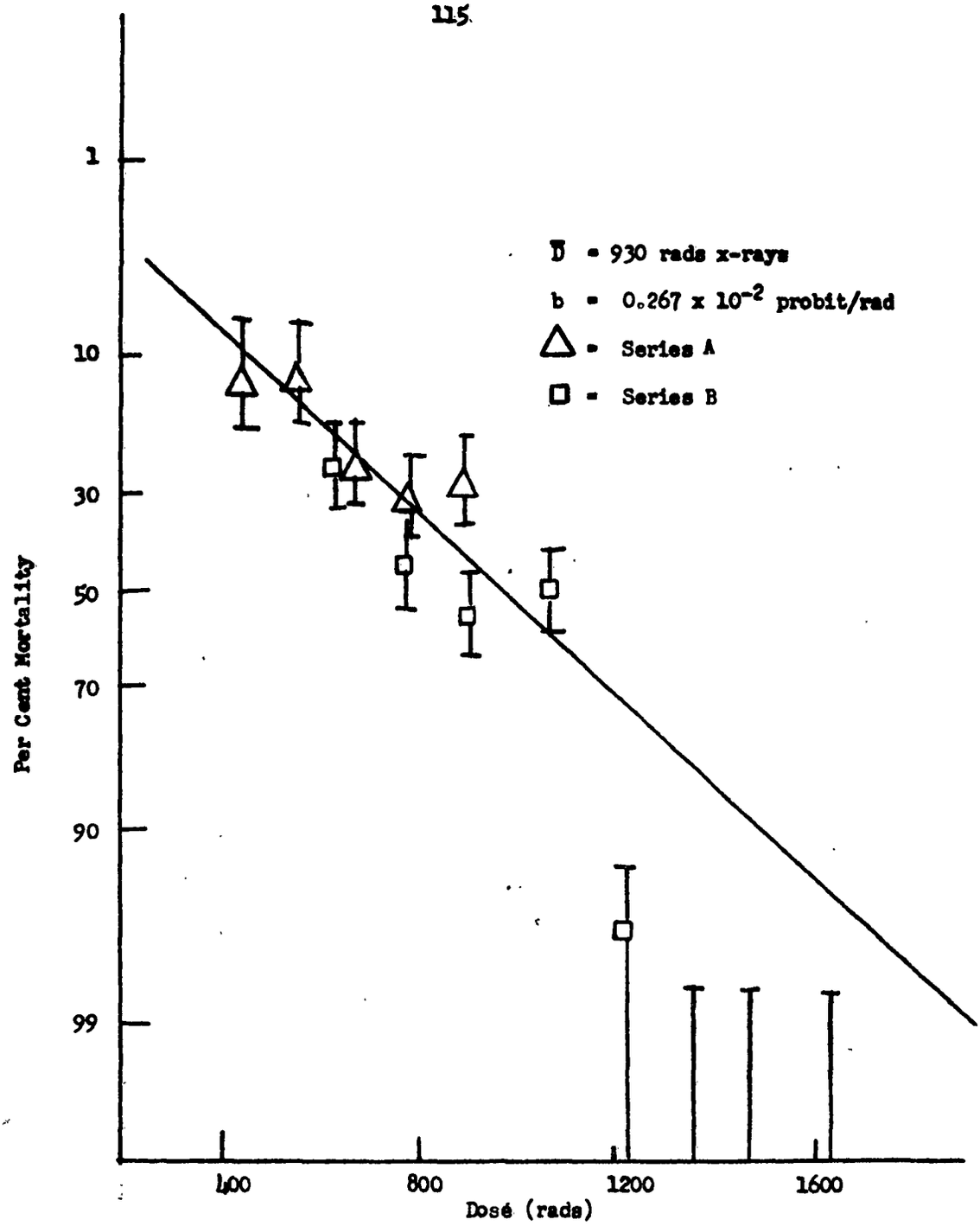

Figure 6. Dose-mortality data for 19 ± 5 weeks old CF, male mice given 30 mgm./kgm. PAPP five minutes prior to whole-body x-irradiation.

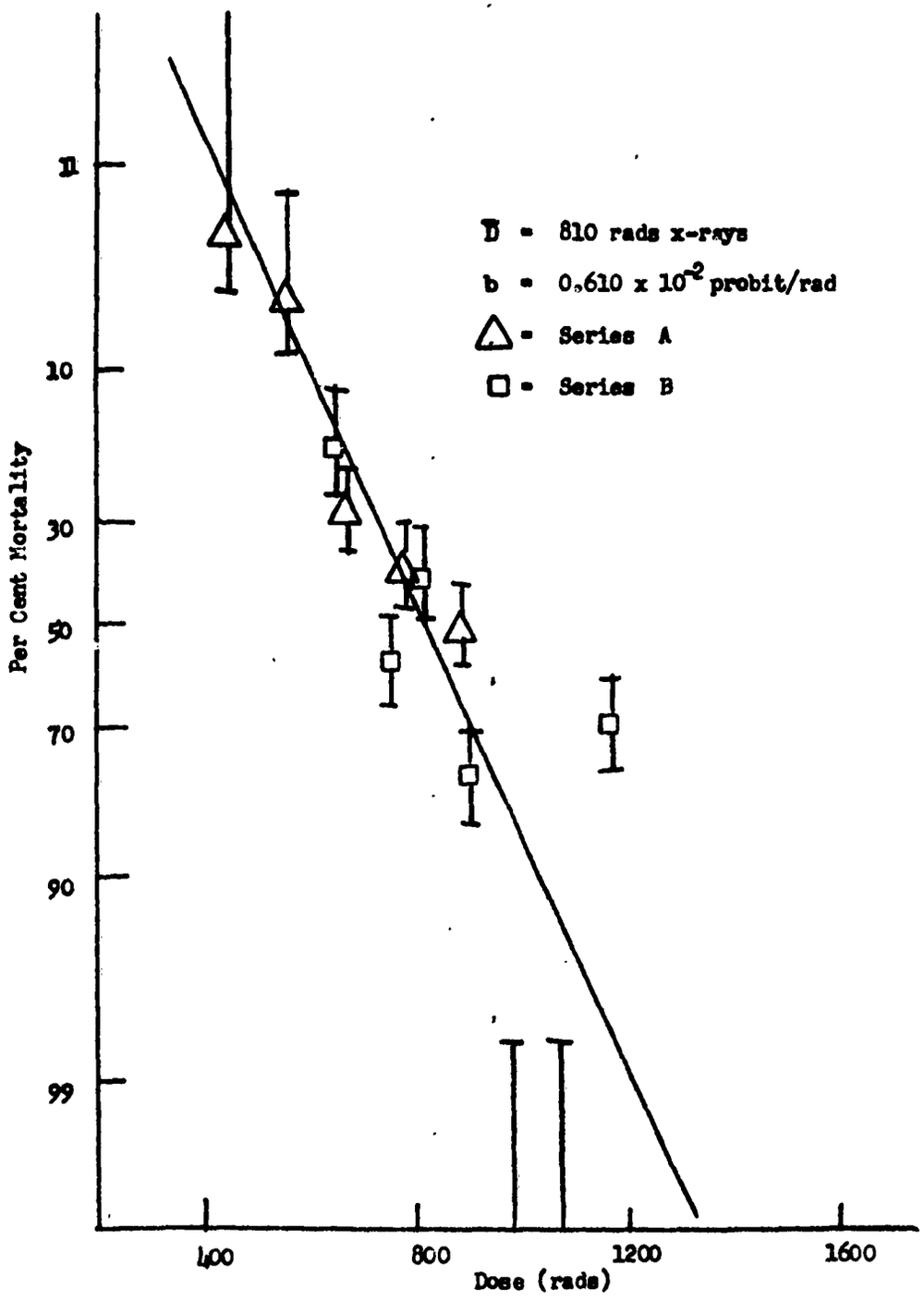

Figure 7. Dose-mortality data for 19 ± 5 weeks old CF_1 male mice given 225 mgm./kgm. MEA five minutes prior to whole-body x-irradiation.

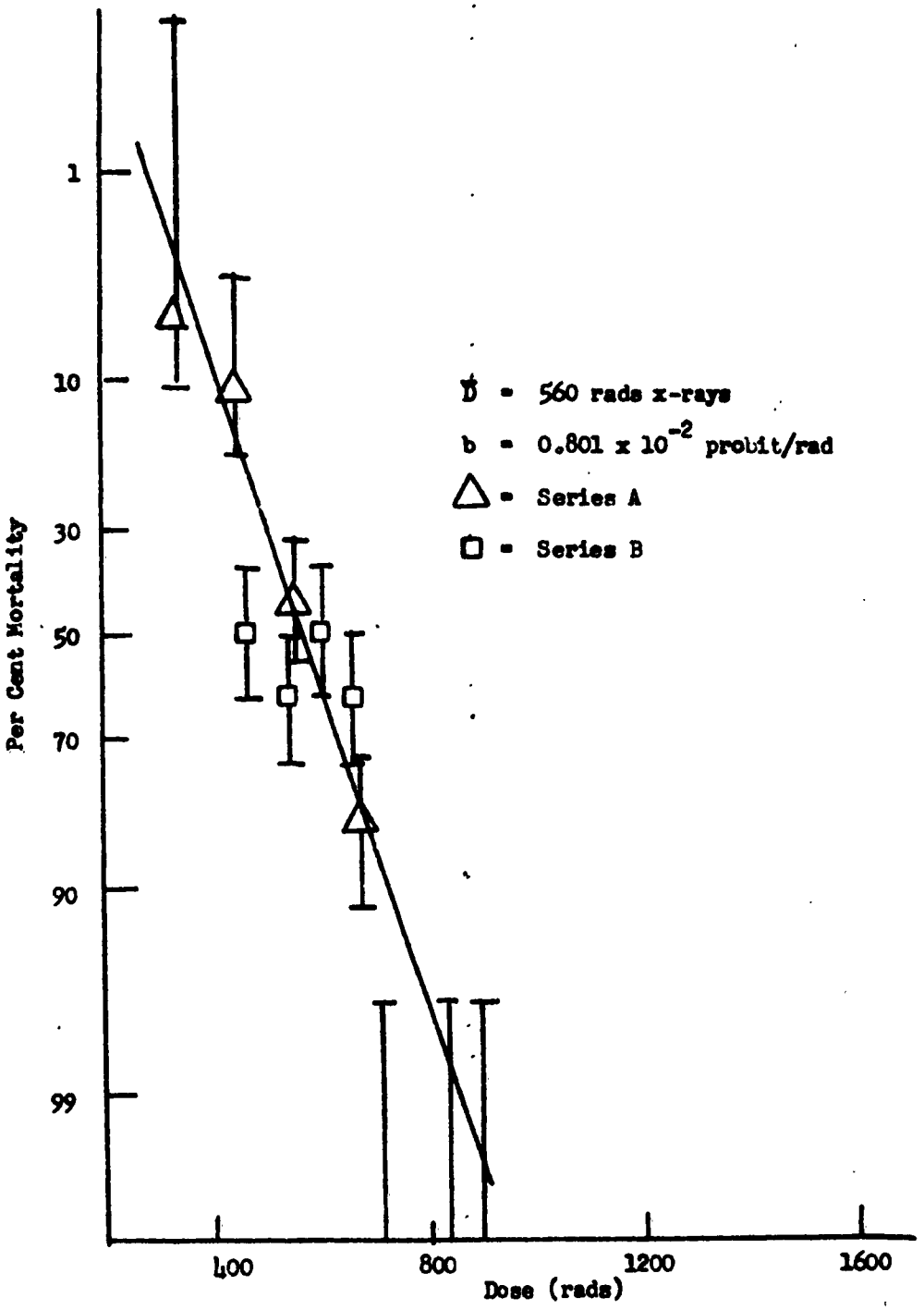

Figure 8. Dose-mortality data for 18 to 19 weeks old CF_1 female mice given vehicle only five minutes prior to whole-body x-irradiation.

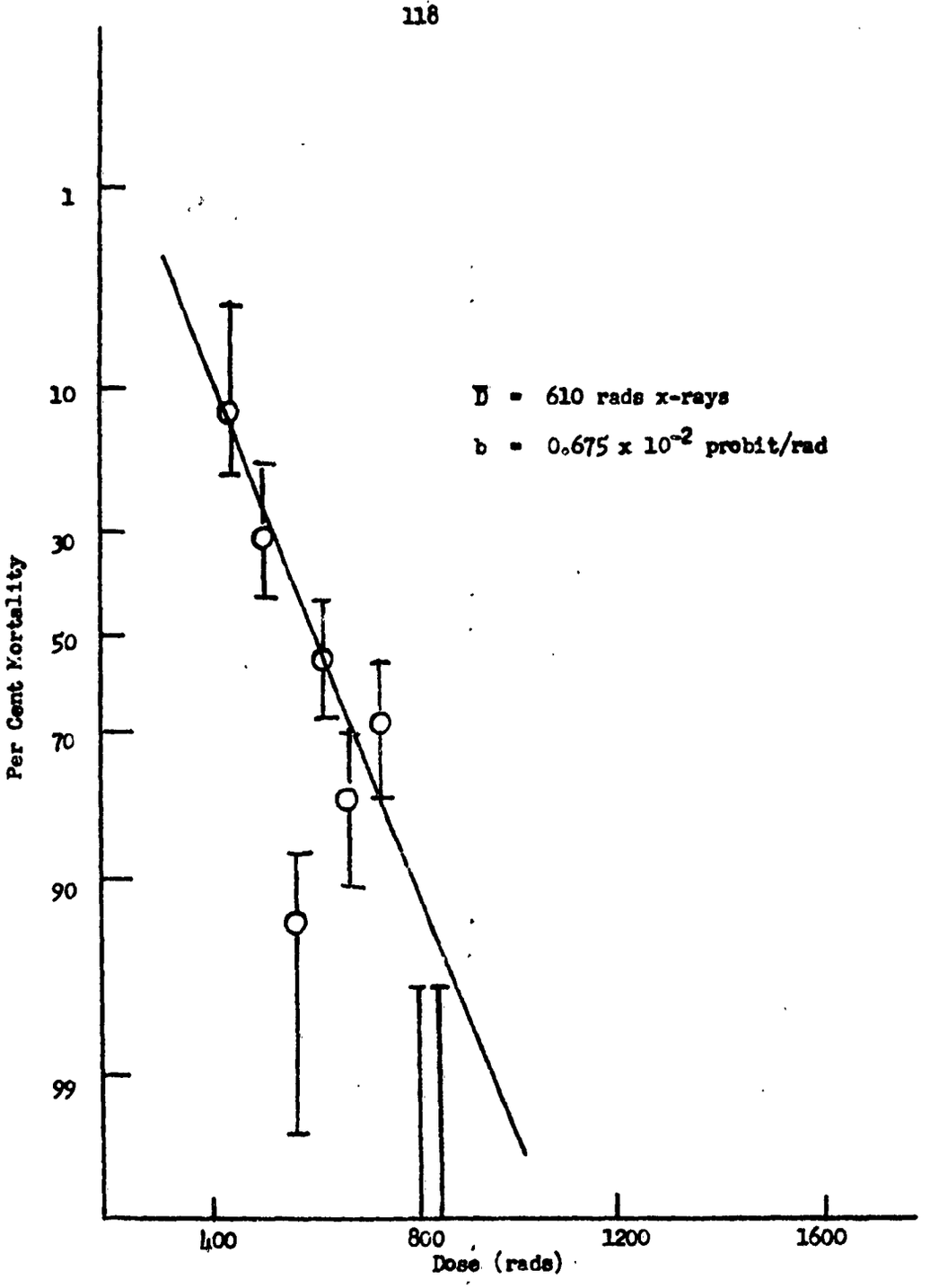

Figure 9. Dose-mortality data for 14 to 15 weeks old CF_1 male mice given vehicle only five minutes prior to whole-body x-irradiation.

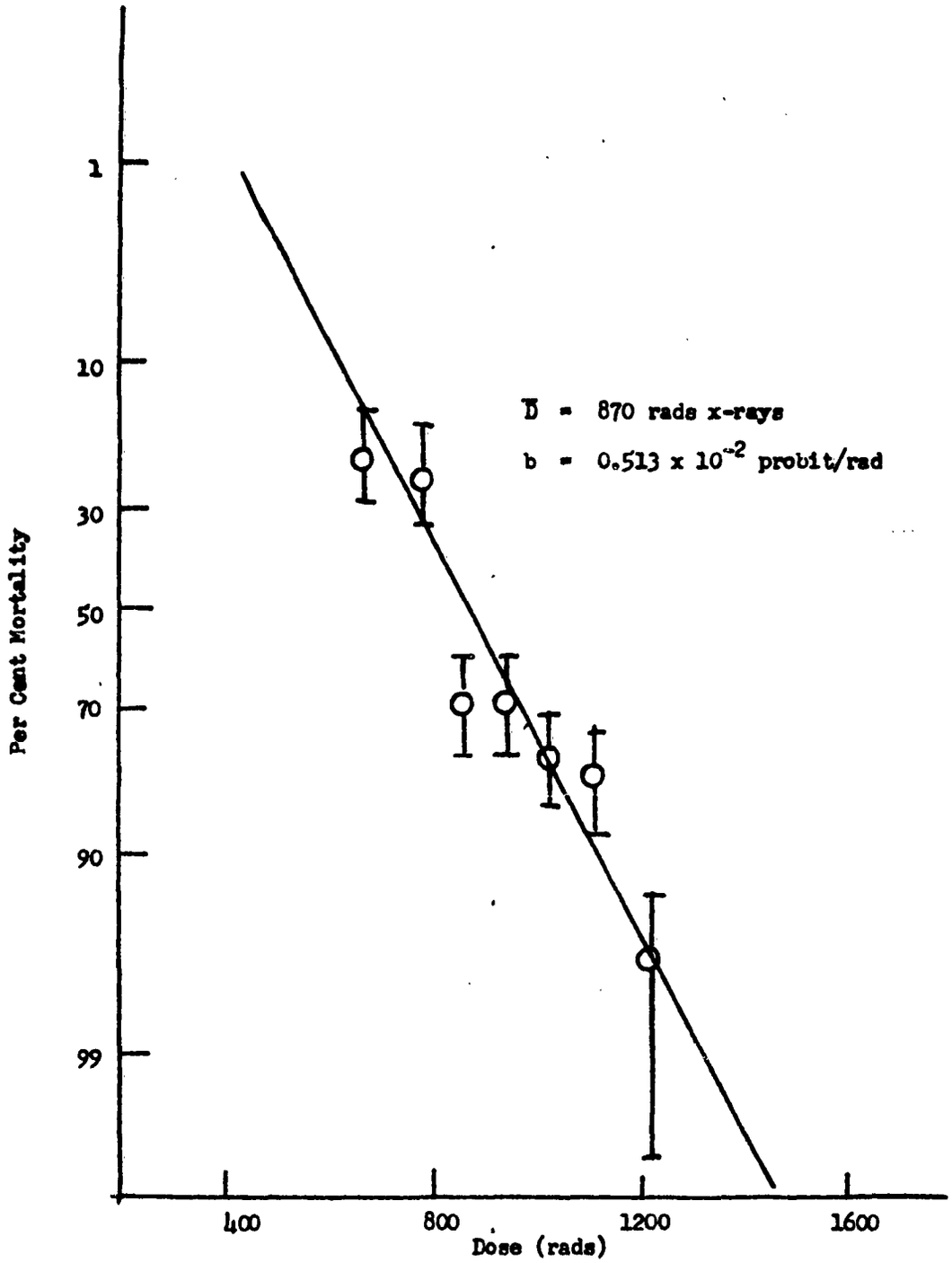

Figure 10. Dose-mortality data for 14 to 15 weeks old CF_1 male mice given 225 mgm./kgm. MEA five minutes prior to whole-body x-irradiation.

For each estimated straight-line fit to the data, the dose producing 50% mortality, $\bar{D}$, and the slope of the line, b, measured in probits per rad, can be obtained. These values are collected in Table 3. Under the assumption that no large errors result from the use of non-identical assay periods, values of DRF (dose reduction factor), RBE (relative biological effectiveness), and RPE (relative protective effectiveness) have been calculated using the data of Table 3. These values are presented in Table 4.

The difference in the efficiency of PAPP and MEA in the concentrations used for protecting against acute lethality as judged from the DRF's and RPE's of Table 4 seems not to be as large as was thought previously. Pending further analysis of the data, the only conclusions that can be drawn at present from the indices are that both PAPP and MEA protect against early lethality in mice irradiated by high-energy protons, and that the degree of protection afforded is roughly the same as that for medium quality x-rays.

The mean dose, $\bar{D}$, for female controls is not greatly different from that of males for either protons or x-rays. The slopes, b, for females, however, are steeper than those for males for both radiations.

The 14 to 15-week old control and MEA groups are seen to give mean doses, slopes, and DRF's quite comparable with those obtained for groups much more heterogenous in age. It may be concluded that there is no bar to using mice in the range 19 ± 5 weeks of age for experiments of this type.

In Table 5 are presented survival data out to 140 days for control (water or 50% aqueous propylene glycol only) mice given various proton doses during the Series A experiments. Also presented are survival data for mice in this series given 30 mgm./kgm. of PAPP or 225 mgm./kgm. of MEA five minutes prior to receiving a proton dose of 939 rads. The quantity q is the fractional number of mice surviving to any selected time, with the same exclusions on immediate and short-term deaths as described above.

The data of Table 5 are plotted in Figures 11 to 19. The straight lines have been fitted to the data by eye on the assumption that the rate of death (-dq/dt) is, in rough approximation, constant over successive intervals of time, the "constant" being different for each interval. Treated in this way, the data exhibit a number of systematic and to some extent anticipated trends. First, the initial rates increase with increasing radiation dose. Second, the initial rate of death is succeeded by a secondary rate of death proportional to, but smaller than, the initial rate. Third, the transition from initial rate to secondary rate occurs earlier in time and at a lower q-value for larger doses. Fourth, for low doses, the initial rate appears to be preceded and followed by periods during which the rate of death is zero--a delay, as it were, occurs before the initial rate appears and also before the secondary rate appears. These delays may be termed initial, secondary, etc. Examining the curves for protected animals, one sees that PAPP-treated animals exposed to 939 rads of protons generate a curve which has the qualitative behavior of that for control animals given 820 rads of protons. On the other hand, MEA-treated animals given 939 rads produce a curve resembling that for control animals receiving 591 rads of protons.

TABLE 3

MID-LETHAL DOSE ($\bar{D}$) AND REGRESSION LINE SLOPE (b) OBTAINED FROM MORTALITY-DOSE CURVES FOR PROTONS AND X-RAYS

	Sex	Ages, Weeks	Drug Group	$\bar{D}$	b, Probits/rad
440 Mev protons	Male	19 ± 5	Control	820	0.291×10^{-2}
	Male	19 ± 5	PAPP	1330	0.178×10^{-2}
	Male	19 ± 5	MEA	1390	0.176×10^{-2}
	Female	18 - 19	Control	680	0.641×10^{-2}
250 Kvp x-rays	Male	19 ± 5	Control	570	0.583×10^{-2}
	Male	19 ± 5	PAPP	930	0.267×10^{-2}
	Male	19 ± 5	MEA	810	0.610×10^{-2}
	Female	18 - 19	Control	560	0.801×10^{-2}
	Male	14 - 15	Control	610	0.675×10^{-2}
	Male	14 - 15	MEA	870	0.513×10^{-2}

TABLE 4

DRF, RBE, AND RPE VALUES OBTAINED FROM MID-LETHAL DOSES OF PROTONS AND X-RAYS FOR MALE MICE 19 ± 5 WEEKS OLD

Radiation / Drug	Protons	X-rays	RBE = 0.70
PAPP	DRF = 1.62	DRF = 1.63	RPE = 1.43
MEA	DRF = 1.70	DRF = 1.42	RPE = 1.72

$$\text{DRF} = \frac{\bar{D}\ (\text{drug})}{\bar{D}\ (\text{control})}; \quad \text{RBE} = \frac{\bar{D}_x\ (\text{control})}{\bar{D}_p\ (\text{control})}$$

$$\text{RPE} = \frac{\bar{D}_x\ (\text{drug})}{\bar{D}_p\ (\text{drug})}$$

TABLE 5

FRACTION OF CF$_1$ MICE SURVIVING VARIOUS DOSES OF PROTON RADIATION VERSUS TIME POST-IRRADIATION

Series A: Males 15 to 19 Weeks Old

Time, Days \ Dose, rads	Controls							PAPP	MEA
	235	351	468	591	702	820	939	939	939
3	1.000	1.000	1.000	1.000	1.000	1.000	1.000	1.000	1.000
6	"	"	"	"	"	.945	.829	.972	.971
9	"	.972	"	.972	.943	.722	.543	.861	.941
12	"	"	.972	.917	.829	.639	.371	.667	.853
15	"	"	.945	.889	.743	.556	"	.611	.735
18	"	"	"	"	.714	.444	.343	"	"
21	"	"	"	.833	.686	"	.286	.583	"
28	"	.861	.917	.778	.514	"	"	.528	"
37	"	"	"	.750	.457	.417	.257	.500	"
42	"	.833	"	.722	"	"	"	.417	.706
49	.971	"	"	.695	"	"	"	.389	.647
56	"	"	.889	.667	"	.389	.229	"	.618
63	.943	"	"	.639	"	"	"	"	"
70	"	"	"	"	"	"	"	.361	"
77	"	"	"	.611	"	"	"	"	"
84	"	.806	"	"	"	"	"	"	"
91	"	"	"	"	"	.361	"	.278	"
100	"	"	.861	"	"	"	"	"	"
105	.914	.778	.833	.583	"	"	.200	"	.588
114	.886	"	.778	"	"	"	"	"	"
121	"	"	"	"	"	.333	"	"	"
126	"	.750	"	"	"	"	.171	"	"
133	"	.695	"	"	"	"	"	"	"
140	.857	"	"	"	"	"	"	"	"

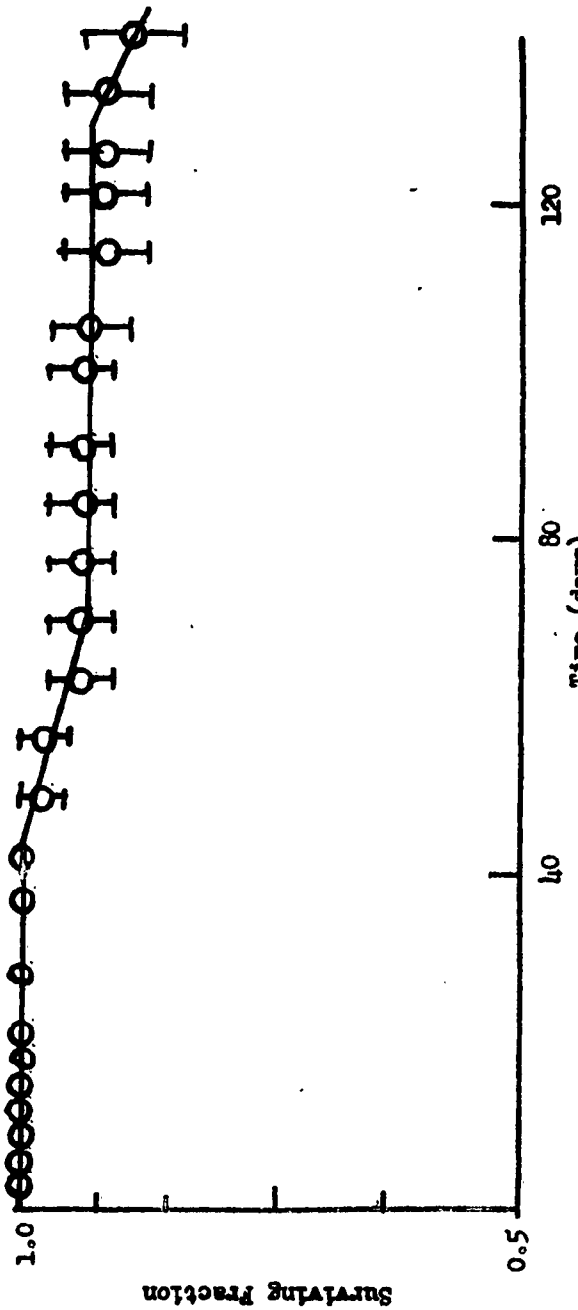

Figure 11. Surviving fraction of CF_1 male mice given vehicle only versus time after 235 rads proton irradiation (Series A).

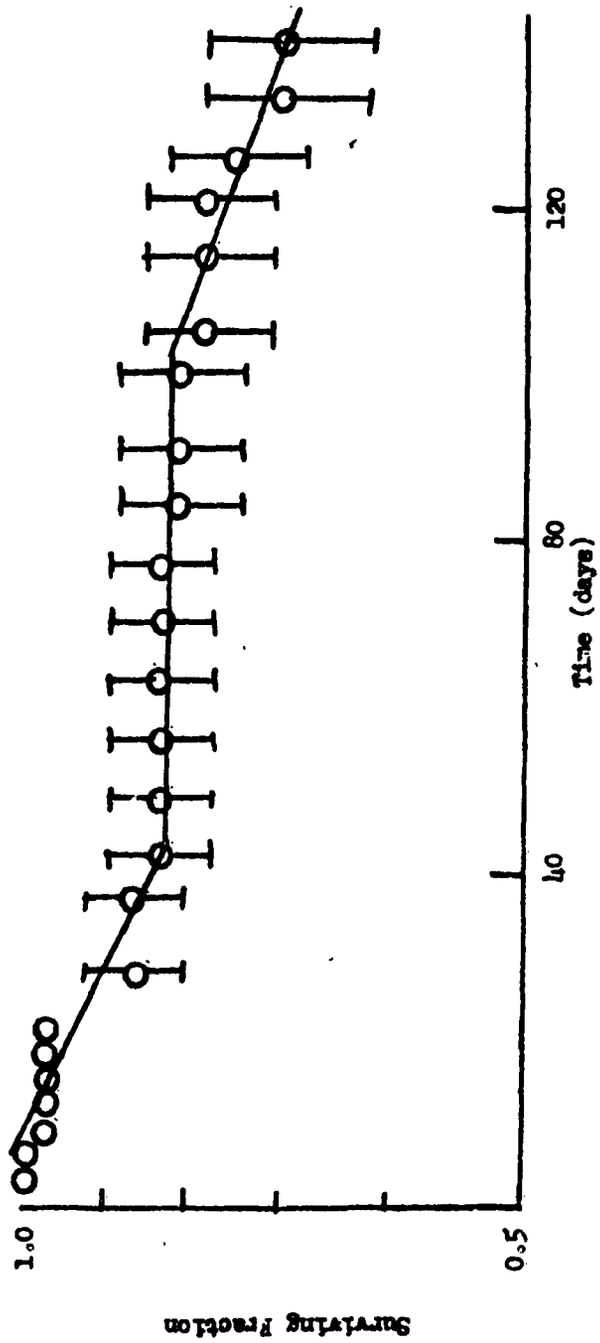

Figure 12. Surviving fraction of CF_1 male mice given vehicle only versus time after 351 rads proton irradiation (Series A).

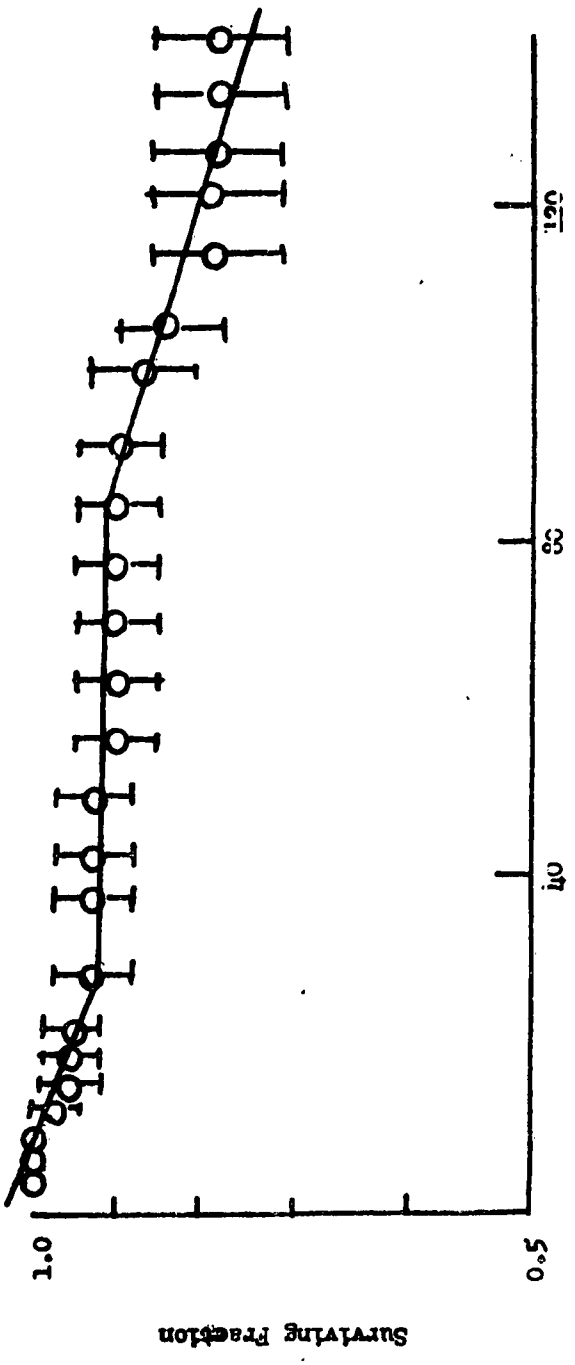

Figure 13. Surviving fraction of CF_1 male mice given vehicle only versus time after 468 rads proton irradiation (Series A).

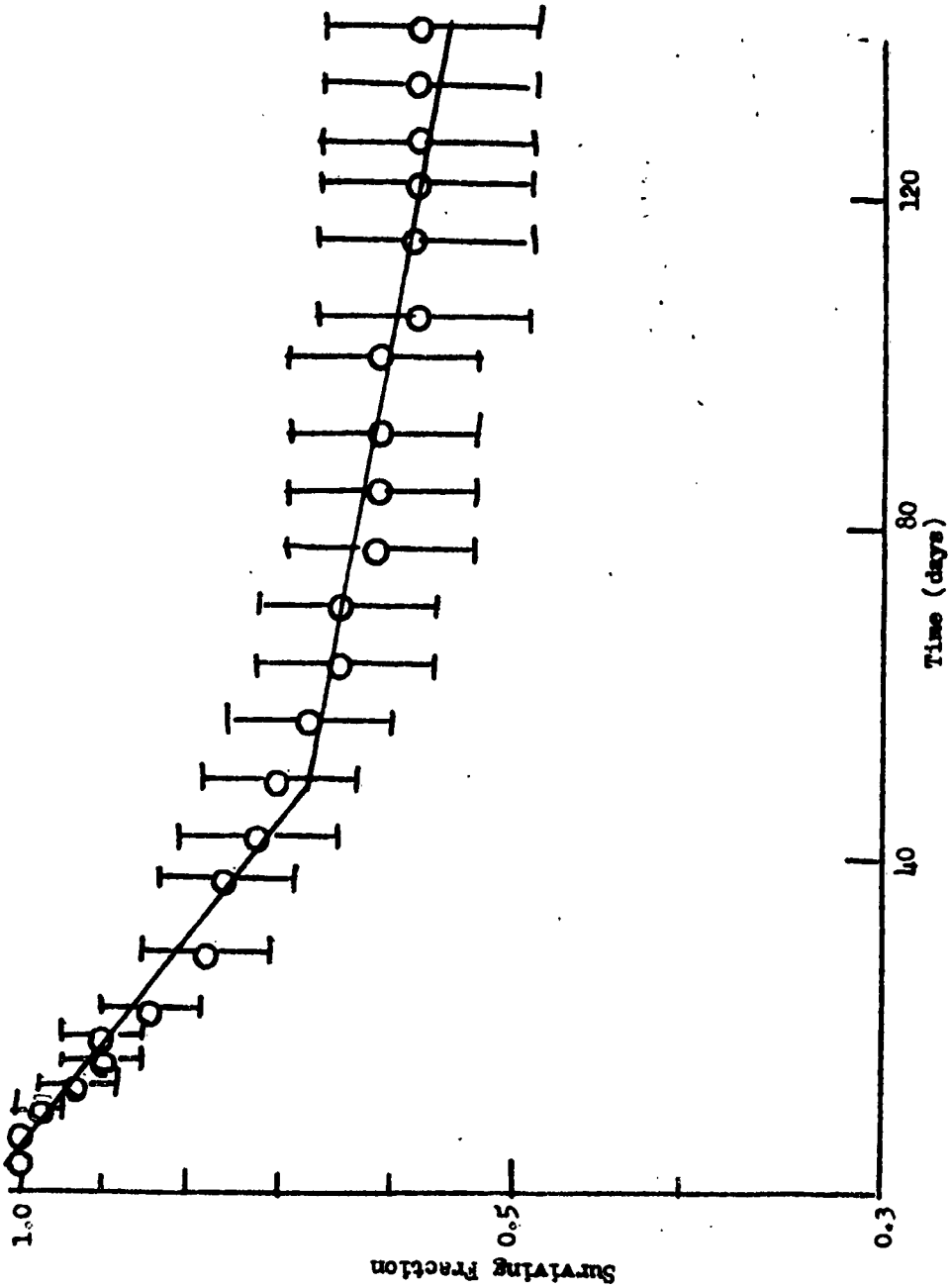

Figure 14. Surviving fractions of CF_1 male mice given vehicle only versus time after 591 rads proton irradiation (Series A).

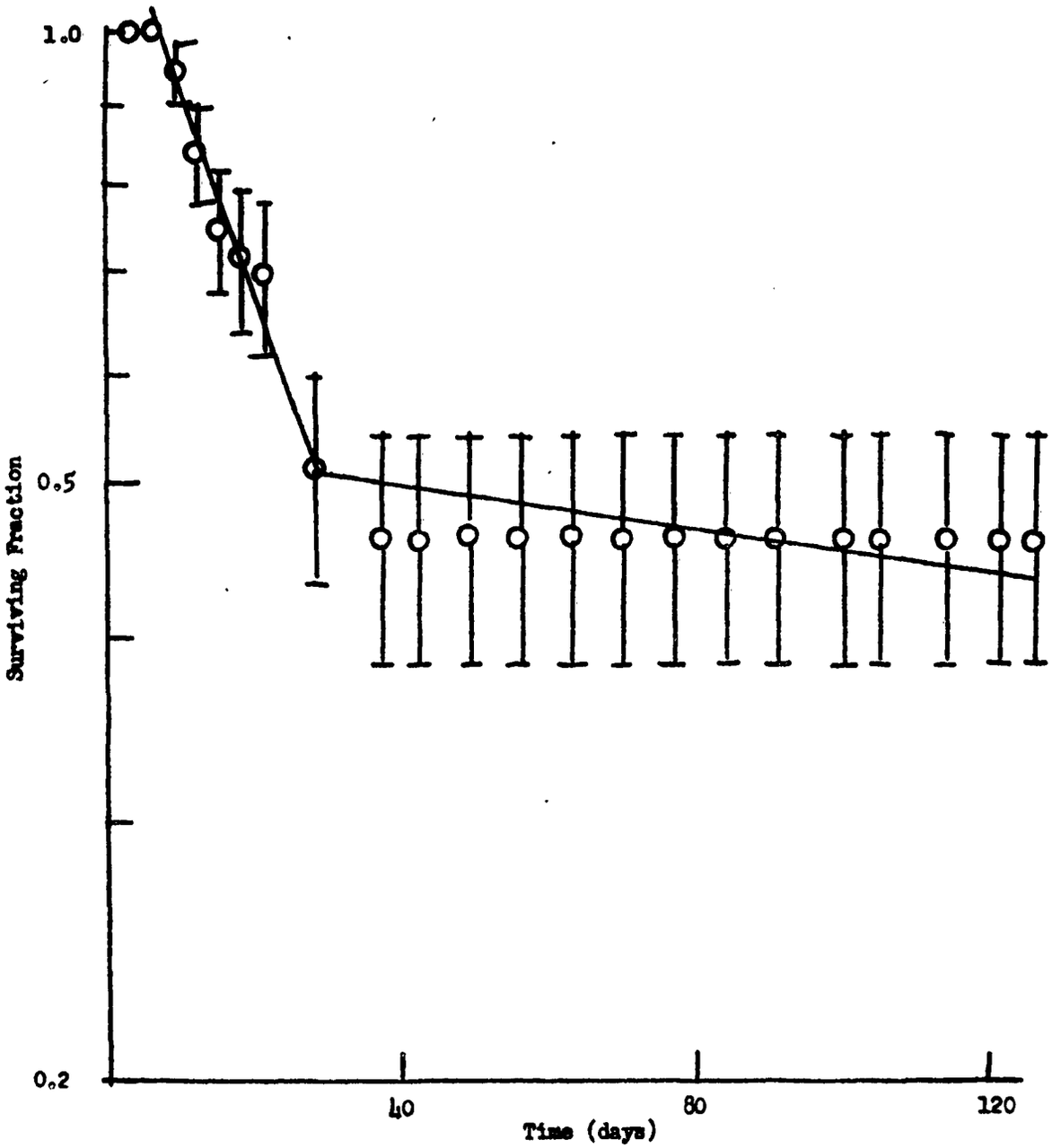

Figure 15. Surviving fraction of CF_1 male mice given vehicle only versus time after 702 rads proton irradiation (Series A).

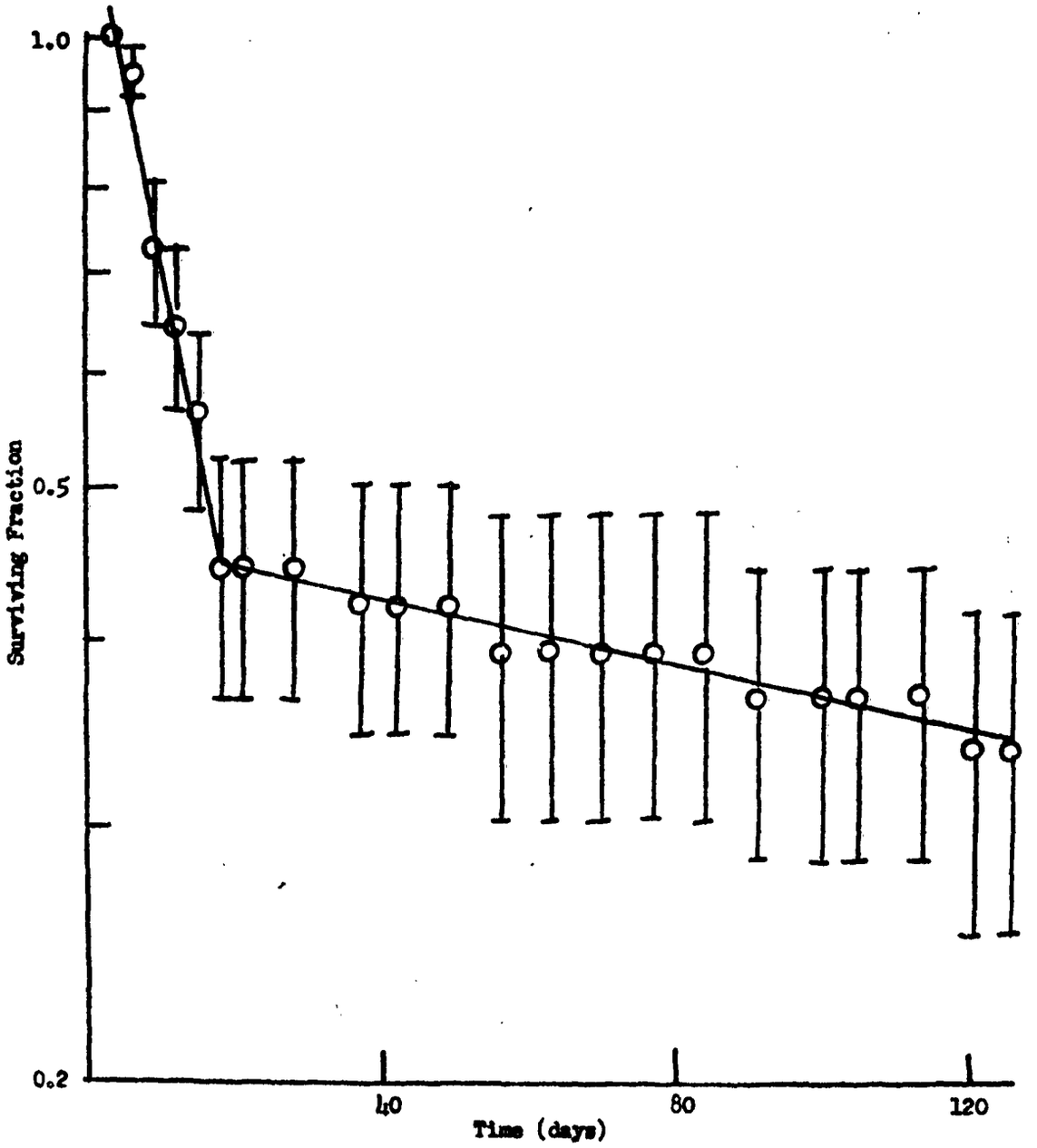

Figure 16. Surviving fraction of CF$_1$ male mice given vehicle only versus time after 820 rads proton irradiation (Series A).

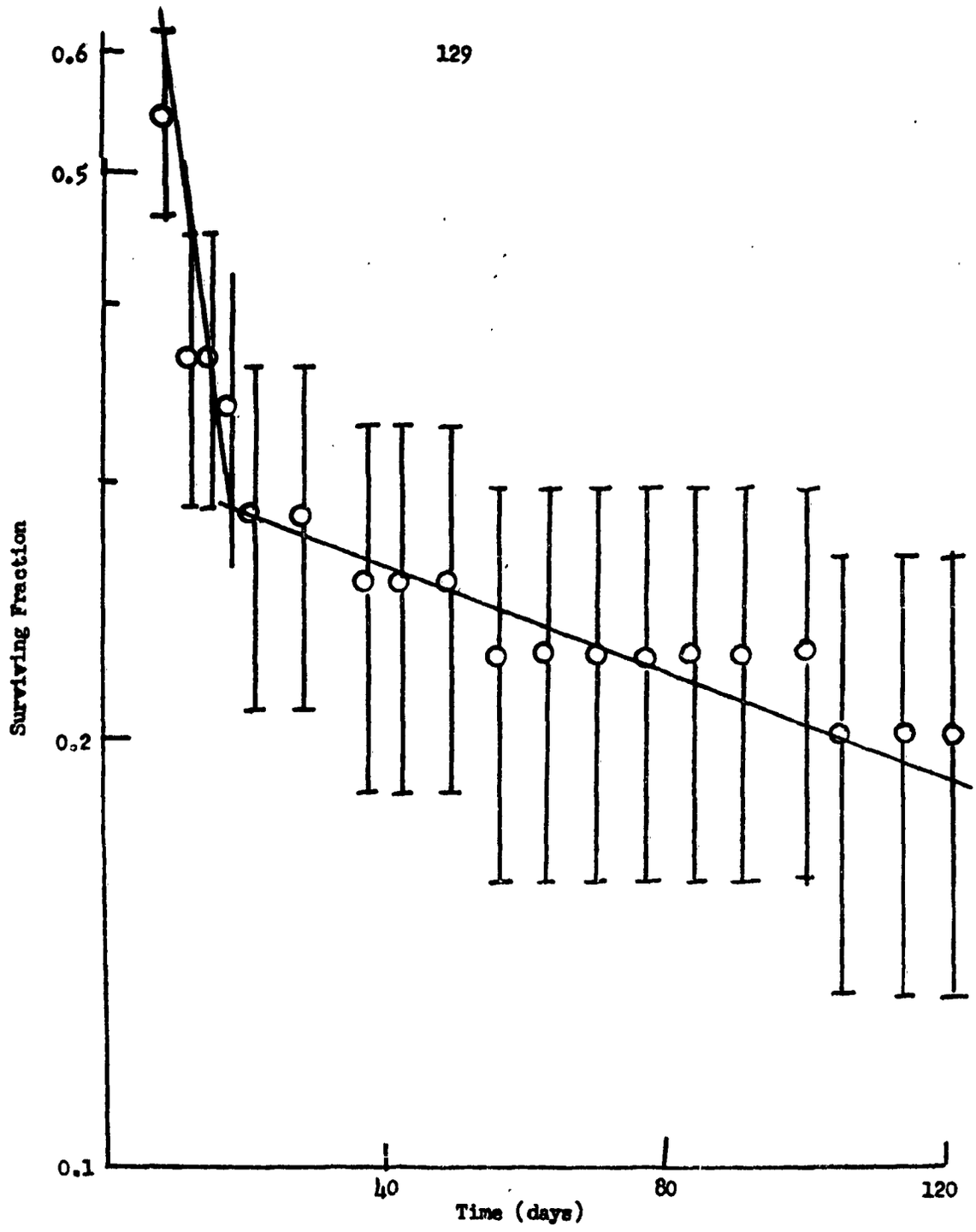

Figure 17. Surviving fraction of CF_1 male mice given vehicle only versus time after 939 rads proton irradiation (Series A).

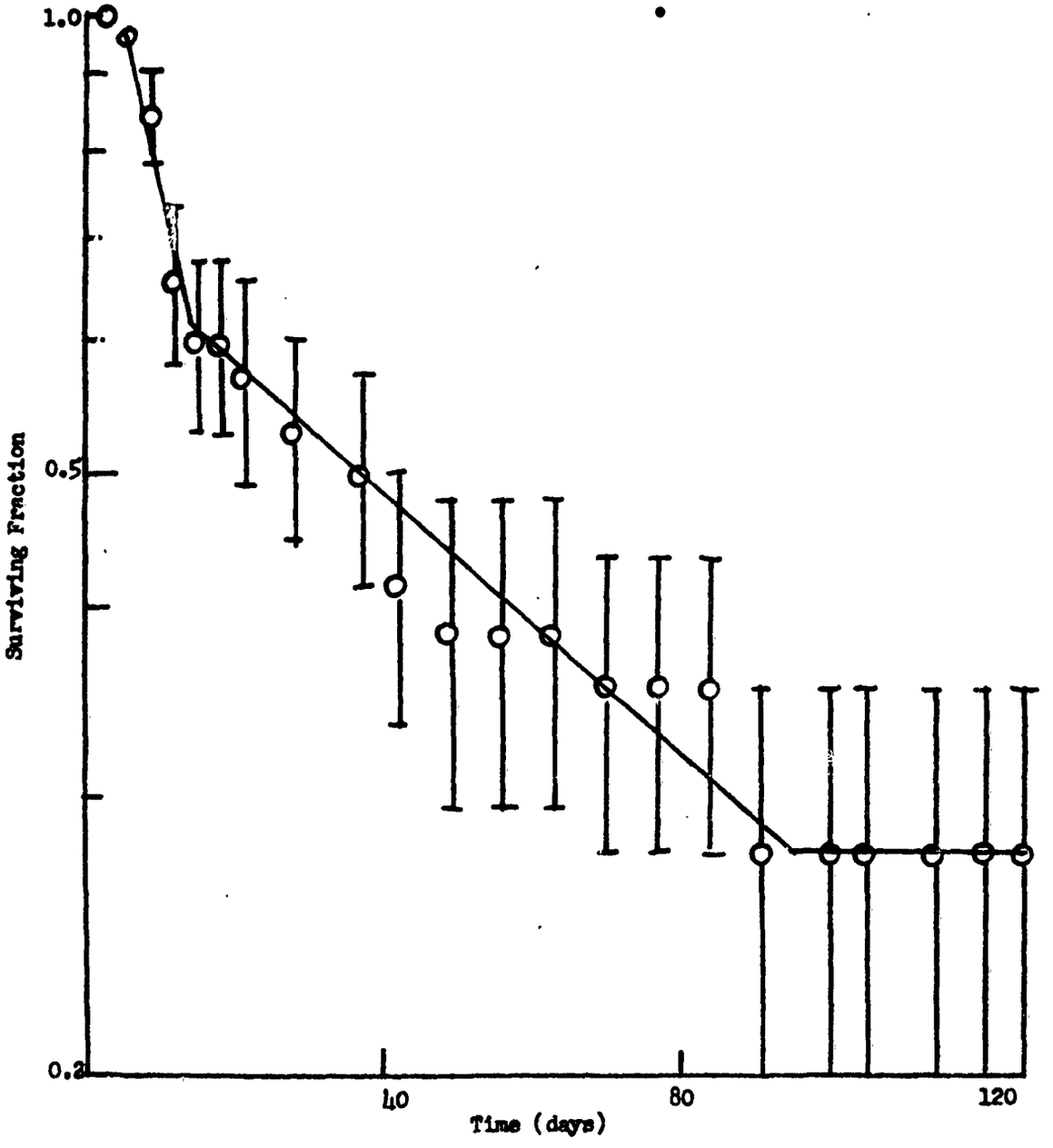

Figure 18. Surviving fraction of CF_1 male mice given 30 mgm./kgm. PAPP versus time after 939 rads proton irradiation (Series A).

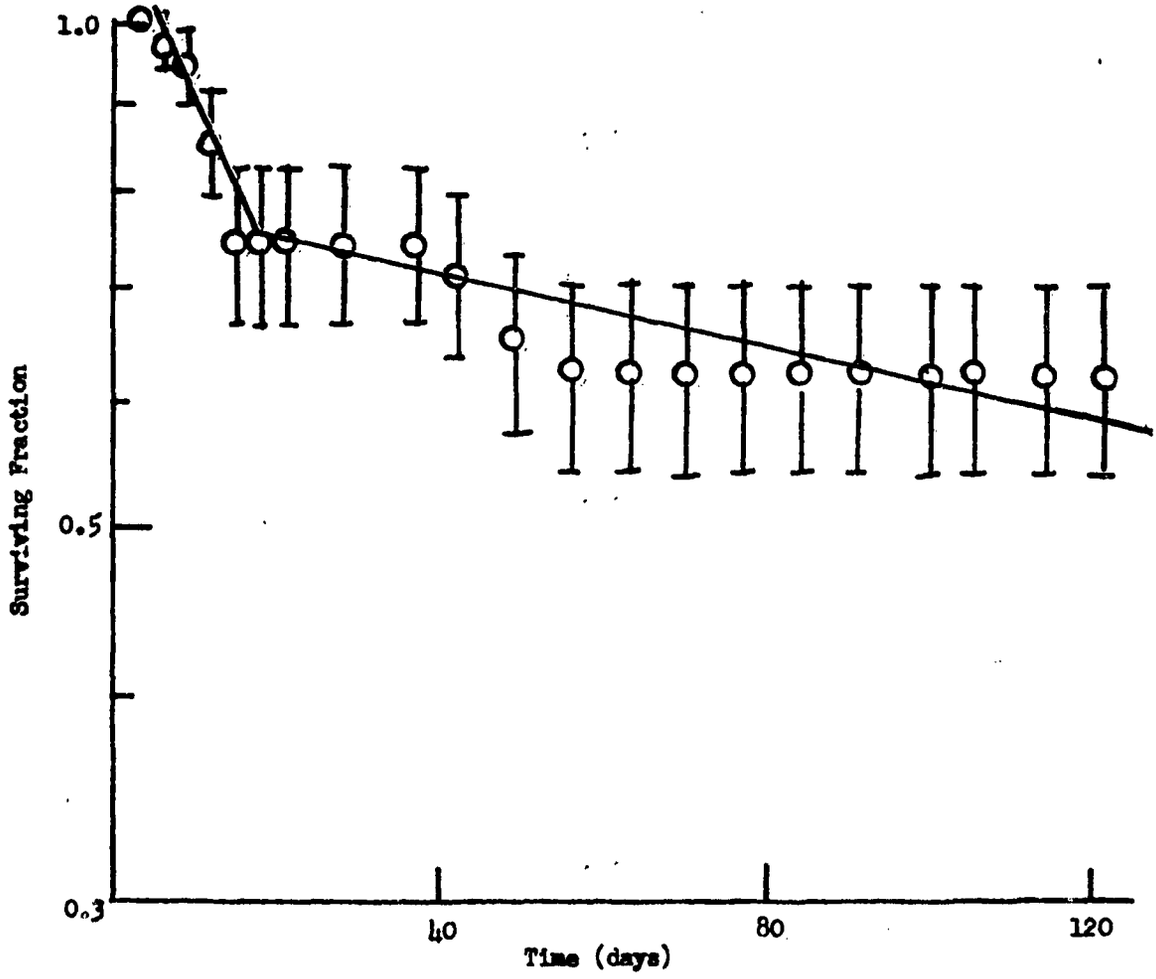

Figure 19. Surviving fraction of CF_1 male mice given 225 mgm./kgm. MEA versus time after 939 rads proton irradiation (Series A).

Discussion

As mentioned previously, the data of Series A and B experiments cannot be fully analyzed for survival until more time has elapsed. The survival data for Series A proton-irradiated animals at 140 days, however, already indicate certain limitations of such response as DRF, RBE, and RPE measured at 30 days. These limitations are probably not peculiar only to the proton data; the latter are used here simply as illustrative.

First, if the initial and secondary rates of death estimated by the lines of Figures 11 to 19 are assumed to indicate the occurrence of two different processes leading to death, the surviving fraction at 30 days, q_{30}, may result from none, one, or both of these processes depending on the dose used. For example, in Figure 11, q_{30} occurs during the initial delay and so is not affected either by the initial or secondary rates of death which follow; in Figure 12, q_{30} results from the occurrence of a portion, but not all, of the initial rate of death; in Figures 16 and 17, q_{30} results from both the initial and a part of the secondary rates of death. Second, and quite apart from any hypotheses as to its cause or nature, the data of Figures 11 to 19 indicate that the rate of death per mouse is still changing at 30 days for most of the doses used, though perhaps less rapidly than at earlier times. The choice of 30 days as the assay time in this system thus appears to have convenience and convention, but not a great deal more to recommend it. Specifically, the significance of the value of q obtained at 30 days to the whole temporal phenomenon of survival after irradiation is obscure.

If the assay point were placed far enough out in time for a plateau in survival to be reached, the significance of such a plateau-q would be much clearer than that of the q_{30}'s. Whether such plateaus exist for this system is not known at present, but this method of assay would presumably require periods of time at least greater than 140 days. For mice of these ages, the behavior of q may begin to depend on aging processes as well as on the experimental stress used, or on some combination of these not easily separable on the basis of the survival data.

Another much used assay point is the time, t_{50}, required for half of the initially treated mice to die. This assay point has about the same limitations on significance and applicability as the q_{30} previously discussed.

These considerations suggest that it may not be possible to adequately describe the survival behavior of irradiated populations of animals using single constants or one-parameter families of curves. In this event, when a description of more complex survival behavior is required, the question arises: How shall radioprotection or other relative effects be evaluated? A method will be proposed here which, to the authors' knowledge, has not as yet been used either in radiobiology or radioprotection studies. The basic notion, however, originated in the population dynamics formulated by Volterra (3). The quantity of interest is the time integral of the fraction of animals surviving to any specified time, which we shall call the survivance,

$$v(t) = \int_0^t q\,dt.$$

Its chief virtue is that it reflects not simply the value of q attained by time t, but the entire time course of the survival process up to time t. Roughly speaking, the survivance is a combined measure of both how many individuals survived and for how long. A measure of the relative effect on survival up to time t of any stress is given by the ratio of survivances to that time of two groups, one stressed, the other not. A measure of the relative effect on survival up to time t of two stresses is given by the ratio of survivances of two groups, each receiving one of the stresses.

A further virtue of the concept of survivance is that it may be expected to correlate more closely with other biological measures of radiation effects than do the conventional indices. As a simplified example, suppose a group of untreated irradiated animals show a q of 1.0 until five days, after which q falls to 0.5 at 30 days; and suppose a group of treated irradiated animals show a q of 1.0 until 15 days, after which q again falls to 0.5 at 30 days. The q_{30} for each of these groups is the same; the t_{50} for these groups is also the same. One would not, however, expect the radiation pathology or biochemistry of either the survivors or of the mice that died to be the same in both groups when it is known that differences in the time course of survival exist. Whether such a measure as survivance would in fact exhibit properties that correlate well with other biological data remains a matter for investigation; the conventional indices, however, would clearly be unable to cope with this type of situation.

Inspection of Figures 17, 18, and 19 shows that at any time up to about 100 days post-irradiation the survivance of MEA-treated mice is greater than that of PAPP-treated mice, which in turn is greater than that of control mice receiving vehicle only. Thus, based on survivance, we would conclude that, in this range of drug and radiation doses, MEA protects more effectively than PAPP against high-energy protons.

Summary

1. Previous studies of radioprotection against 440 Mev proton radiation have been replicated and extended confirming the original conclusion that pre-irradiation treatment with MEA and PAPP are able to reduce 30-day mortality in CF_1 mice.

2. A new assay parameter, survivance, defined as the time integral of the fractional number of animals surviving to a given time, is introduced and several of its properties and intended uses discussed qualitatively.

3. Judged on the basis of the survivance at 100 days, MEA protects more effectively than PAPP against 440 Mev protons.

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THE INFLUENCE OF EXPOSURE TO LOW LEVELS OF GAMMA OR FAST NEUTRON IRRADIATION ON THE LIFE SPAN OF ANIMALS

II. Gross and Microscopic Pathology in Mice Given Chemical Radioprotective Agents Prior to Irradiation with 440 Mev Protons

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This report concerns: Autopsy and histopathological findings in the tissues of CF_1 male mice given 2-mercaptoethylamine (MEA) or p-aminopropiophenone (PAPP) prior to whole-body irradiation with high-energy (440 Mev) protons or x-rays.

Immediate or ultimate application of the results: Information is needed concerning the relative biological effectiveness of high-energy protons in mammalian systems. Having begun studies to obtain information of this type, it was advantageous to concurrently evaluate the ability of chemical radioprotective agents to prevent damage to such systems. In addition to the practical value of such studies, they are of considerable theoretical interest with regard to the relationship of LET to radiation injury and to the mechanism of action of the chemical radioprotective agents. The studies described in this report constitute part of a program designed to provide information on the biologic effects of high-energy proton irradiation, protection against these effects, and a comparison with results obtained using radiations having other LET values (10-50 Mev electrons, relativistic neutrons, lower energy protons, etc.).

* * * * * * * *

Previous studies describing histopathological findings in the tissues of proton-irradiated animals have been designed primarily to investigate the therapeutic value of such radiation in the treatment of malignancies and for radiosurgery. As a result, the information obtained concerns mainly the effects of partial-body localized irradiation rather than of whole-body exposure. The possible uses of the unique properties of high energy protons, deuterons, and alpha particles in oncology, neurology, etc. have been discussed by Wilson, Tobias, Falkmer, and others (1-7) in reports which include descriptions of the pathologic effects of proton beam irradiation of the pituitary, hypothalmus, and of other parts of the central nervous system. A comparison of the cataractogenic effects of protons and alpha particles in the monkey has been provided by the studies of Zellmer and Allen (8) who conclude that the RBE for cataractogenesis is about 2.0 for proton irradiation and about 1.0 for alpha radiation relative to cobalt-60 gamma radiation. Warshaw and Oldfield (9) found that 90 Mev protons are about two times as effective as 250 Kvp x-irradiation in reducing the weight of the spleen and thymus of whole-body irradiated IAF_1 mice. The only other whole-body proton irradiation studies with which we are familiar are those of Tobias, Anger, and Lawrence (10) who used strain A mice exposed

to 315 Mev protons and Kurlyandyskya and coworkers (11) who irradiated white male mice with 660 Mev protons. Because of the apparent scarcity of information concerning the histological effects of whole-body proton irradiation in animals, it was of interest to carry out both gross and microscopic examination of the tissues of mice exposed to 440 Mev protons in one of the current programs of this laboratory.

The studies presented in this report comprise the initial observations made in those mice dying within 30 days after exposure to either protons or x-rays. A relatively small number of the animals exposed to each of the dose levels of either protons or x-rays have been examined and only those which were exposed to the higher radiation dose levels are presented in this report. Additional studies have been carried out in which groups of mice exposed to either 440 Mev protons or 250 Kvp x-rays have been serially sacrificed during the initial post-irradiation period, and it is planned to extend these studies to include an investigation of the pathogenesis of some of the delayed effects of exposure to the two types of radiation.

Materials and Methods. Detailed descriptions of the physical and biological methods employed in the irradiation of the animals have been presented in a previous report (12). All of the mice used for the present study were CF$_1$ adult males (15 to 19 weeks of age) selected from several shipments, randomized, and assigned to the various protected or control groups shown in Table 1. Only a small fraction of the animals exposed at each dose level were included in these studies and only those dose levels which produced appreciable mortality within the 30 days after the radiation exposure have been investigated. The tissues of irradiated mice were obtained from animals which died during the first 30 days after radiation exposure or which were sacrificed in an obviously moribund condition. During the post-irradiation period, the cages were inspected twice daily for dead mice and autopsies were carried out on all of the dead animals except where post-mortem autolysis made this impractical. The following tissues were routinely taken for the histological examination: liver, kidney, spleen, heart, lungs, thymus, testis, lymph nodes (mediastinal and mesenteric), duodenum, pancreas, and sternum. The tissues were fixed in neutral buffered formalin, embedded with paraffin, and stained routinely with hematoxylin and eosin. Frozen sections of the liver of most of the animals were prepared and stained with Oil Red O for fat. Sections of some of the hearts were stained with Prussian Blue for hemosiderin and occasional sections of the liver, kidney, or lung were stained with methylene blue for bacteria. Several sections of the spleen from these animals and some of the bone marrow sections were stained with Azure-eosinate.

Results

Gross pathologic findings in mice exposed to whole-body proton or x-radiation and the influence of pre-irradiation administration of radioprotective agents on these findings. The major gross pathologic findings in the proton-irradiated mice are summarized in Table 2. The most frequent pathologic finding at autopsy in these animals was hemorrhage in the form of petechiae or echymosis involving rather large areas of the affected organs. The organs most frequently involved were the lungs, testis, brain, lymph nodes, and adrenal glands. Hemorrhages were also observed in various parts of the gastrointestinal tract (including the liver), in the urinary bladder, kidney, abdominal subcutaneous

TABLE 1

NUMBER OF ANIMALS EXAMINED AND MEAN TIME OF DEATH[a] FOR VARIOUS PROTON DOSES AND CHEMICAL PROTECTORS

Pre-irradiation Treatment	Proton Radiation Dose			
	Group I 0 rad	Group II 939 rads	Group III 820 rads	Group IV 702 rads
Propylene glycol plus H_2O	5 11[b]	7 8	6 11	1 9
Water	5 11	6 9	3 11	6 16
MEA	5 11	6 10	2 11	2 18
PAPP	5 11	6 17	3	2 11
None	5 11	..	..	..

[a]Or sacrifice.

[b]Days post-injection.

TABLE 2

FREQUENCY AND SEVERITY OF MAJOR GROSS PATHOLOGIC FINDINGS IN PROTON-IRRADIATED MICE

Major Gross Pathologic Findings	Chemical Protectors	Proton Radiation Doses			
		Group I 0 rad	Group II 939 rads	Group III 820 rads	Group IV 702 rads
Hemorrhagic foci in liver and lungs	Propylene glycol and water	0/5	3/7 b,b,b	2/6 a,b	0/1
	Water	0/5	4/6 a,a,a,b	1/3 a	2/6 a,a
	MEA	0/5	2/6 a,b	2/2 a,a	1/2 a
	PAPP	0/5	2/6 a,a	1/3 a	
	None	0/5	...	...	...
Hemorrhages in the testis and sometimes mesenteric lymph node, adrenal, bladder, G.I.T. cerv. l.n., kidney	Propylene glycol and water	0/5	2/7 a,d	3/6 a,b,c	1/1 b
	Water	0/5	2/6 b,b	1/3 a	3/6 a,b,b
	MEA	0/5	2/6 b,b	1/2 a	0/2
	PAPP	0/5	3/6 b,b,b	1/3 a	1/2 a
	None	0/5	...	...	...
Patchy hemorrhages in the brain	Propylene glycol and water	0/5	1/7 b	3/6 a,a,b	0/1
	Water	0/5	1/6 a	0/3	1/6 a
	MEA	0/5	0/6	1/2 a	0/2
	PAPP	0/5	2/6 a,a	1/3 a	1/2 a

a = mild, b = moderate, c = makred, d = very marked

TABLE 2--Continued

Major Gross Pathologic Findings	Chemical Protectors	Proton Radiation Doses			
		Group I 0 rad	Group II 929 rads	Group III 820 rads	Group IV 702 rads
Fatty livers	Propylene glycol and water	0/5	1/7 b	1/6 a	0/1
	Water	0/5	0/6	0/3	3/6 a,a,a
	MEA	0/5	1/6 b	2/2 a,a	0/2
	PAPP	0/5	1/6 a	2/3 a,a	...
	None	0/5	...	...	...
Necrotic liver	Propylene glycol and water	0/5	1/7 a	0/6	0/1
	Water	0/5	0/6	0/3	1/6 a
	MEA	0/5	0/6	0/2	0/2
	PAPP	0/5	0/6	0/3	0/2
	None	0/5	...	...	...

tissues and even in the heart of a few animals given the highest doses of whole-body proton radiation. Another frequent finding was moderate to marked fatty change in the liver. One of the animals in each of the high dose level groups had pin-point grayish nodules in the liver. There was a soft stone in the urinary bladder of one mouse given the highest dose of proton radiation and an abscess-like lesion was seen in the ophthalmic lobe of the brain in a mouse from the group given 702 rads of protons.

The major gross pathologic findings in the x-irradiated mice are summarized in Table 3. Hemorrhages similar to those seen in the proton-irradiated animals were seen in many of the x-rayed mice. The organs most frequently involved were the brain, lungs, testis, and kidneys. Fatty livers, mostly of mild degree, were also observed occasionally and one animal had pin-point whitish spots in the liver.

The spleen and testis weights for non-irradiated groups of mice given the various pre-irradiation treatments 11 days before sacrifice have been compared with the values from the animals given 939 rads of proton radiation. The results are presented in Figures 1 and 2. It can be seen that at death, both spleen and testis weights are depressed relative to those of non-irradiated animals. The fractional decrease found is greater for spleen than for testis and there is no marked indication of any reduction in weight loss due to the administration of the protective substances.

Microscopic findings in the tissues of mice exposed to whole-body proton or x-radiation with or without prior treatment by chemical radioprotective agents. The results of the microscopic examination of the tissues of the animals included in these studies are presented in Tables 4 and 5. The major findings were seen in the spleen, bone marrow, liver, and testis.

Spleen. There was mild to marked atrophy of the lymphoid tissue in all of the groups exposed to high-energy protons and the severity of these changes appeared to be related to the radiation dose administered. An interesting finding was the apparent faster recovery of the hematopoietic system in the animals given the radioprotective agents prior to the proton exposure. The recovery of the cells in the myeloblastic and erythroblastic series appeared to be more rapid in the mice given either the MEA or the PAPP prior to exposure. Congestion of the spleen was marked in several animals at each dose level of proton radiation. Extramedullary hematopoiesis was present in the spleens of all non-irradiated control mice in marked to moderate degree. Extramedullary hematopoiesis was absent or markedly reduced in all of the non-protected proton irradiated animals, but was present in the mice protected by either MEA or PAPP. Hemosiderosis was noted in all of the animals examined and did not appear to be related in degree to the radiation exposure.

In the x-rayed mice, there was atrophy of the lymphoid tissue but this was less marked in the mice given the radioprotective agents prior to the x-ray exposure. The mice treated with PAPP and MEA exhibited atrophy of the lymphoid tissue also, but recovery of the hematopoietic cells in these animals (particularly of the granulocytic series) appeared to be stimulated. The non-protected x-rayed mice exhibited moderate congestion of the spleen. The non-protected mice exhibited less extramedullary hematopoiesis in the spleen than the mice given either MEA or PAPP. The pre-irradiation administration of the

TABLE 3

FREQUENCY AND SEVERITY OF MAJOR GROSS PATHOLOGIC FINDINGS IN X-IRRADIATED MICE

Major Gross Pathologic Findings	Chemical Protectors	X-radiation Doses			
		Group I 0 rad	Group II 888 rads	Group III 777 rads	Group IV 666 rads
Patchy hemorrhages in the brain	PG + H_2O	0/5	...	...	2/4 a,a
	H_2O	0/5	...	...	2/6 a,a
	MEA	0/5	4/8 a,a,a,b	0/7	0/2
	PAPP	0/5	1/5 a	2/5 a,a	2/3 a,a
	None	0/5	...	...	...
Hemorrhages in the testis	PG + H_2O	0/5	...	...	1/4 a
	H_2O	0/5	...	...	1/6 a
	MEA	0/5	3/8 a,a,b	2/7 a,a	0/2
	PAPP	0/5	2/5 a,a	0/5	0/3
	None	0/5	...	...	...
Hemorrhages in lungs and kidney	PG + H_2O	...	...	...	3/4 a,a,a
	H_2O	...	...	...	1/6 a
	MEA	0/5	1/8 a	3/7 a,a,a	1/2 a
	PAPP	0/5	2/5 a,a	3/5 a,a,a	2/3 a,a
Fatty livers	PG + H_2O	...	...	...	1/4 a
	H_2O	...	...	...	1/6 a
	MEA	0/5	1/8 a	1/7 a	0/2
	PAPP	0/5	1/5 a	0/5	1/3 a

a = mild, b = moderate, c = marked, d = very marked.

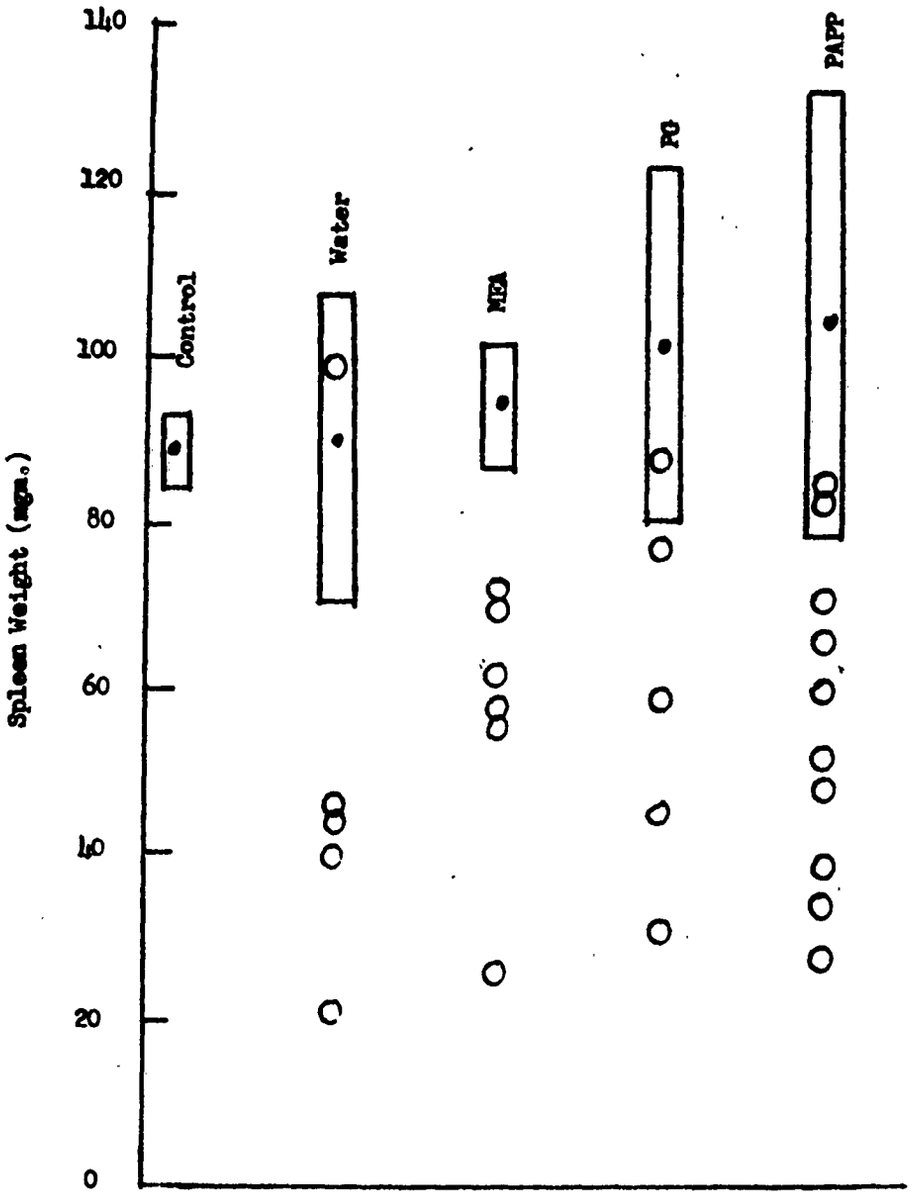

Figure 1. Spleen weights for unirradiated mice sacrificed at 11 days post-injection, ⊡ , and for mice not surviving 939 rad protons, ○ . Standard deviation, □ , of individual values from mean shown for unirradiated mice.

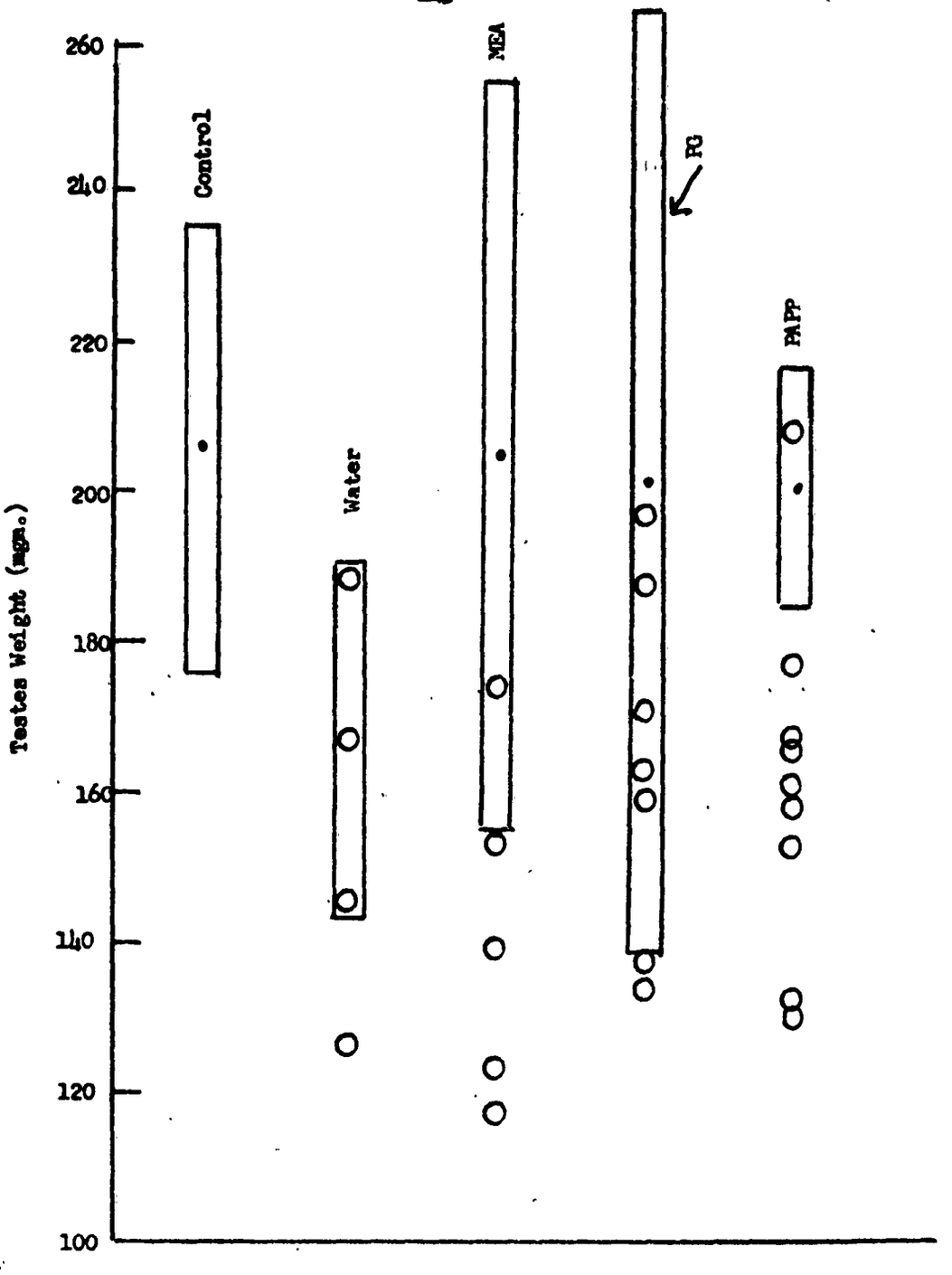

Figure 2. Testes weights for unirradiated mice sacrificed at 11 days post-injection, ⊡ , and for mice not surviving 939 rad protons, ○ . Standard deviation, □ , of individual values from mean shown for unirradiated mice.

TABLE 4

FREQUENCY AND SEVERITY OF MAJOR HISTOPATHOLOGIC FINDINGS IN PROTON-IRRADIATED MICE

Major Histopathologic Findings	Chemical Protectors	Proton-Radiation Doses			
		Group I 0 rad	Group II 939 rads	Group III 820 rads	Group IV 702 rads
Atrophy of the lymphoid tissue	PG + H_2O	0/5	7/7 b,b,c,c,c,c,c	5/6 b,b,c,c,c	1/1 b
	H_2O	0/5	6/6 b,c,c,c,c,d	3/3 a,b,b	3/6 a,a,b
	MEA	0/5	5/6 a,b,b,c,c	2/2 a,b	1/2 b
	PAPP	0/5	5/6 a,a,b,b,c,c	3/3 b,b,a	2/2 a,b
	None	0/5	...	...	...
Recovery of hematopoietic cells, particularly myeloblastic	PG + H_2O	0/5	0/7	1/6 c	0/1
	H_2O	0/5	0/6	0/3	2/6 a,b
	MEA	0/5	4/6 a,a,b,b	1/2 c	1/2 d
	PAPP	0/5	2/6 b,d	1/3 a	2/2 a,a
	None	0/5	...	...	...
Congestion of the spleen	PG + H_2O	0/5	2/7 a,c	2/6 a,c	0/1
	H_2O	0/5	0/6	0/3	1/6 a
	MEA	0/5	0/6	0/2	0/2
	PAPP	0/5	0/6	0/3	0/2
	None	0/5	...	...	...
Extramedullary hematopoiesis	PG + H_2O	5/5 b,b,c,c,c	1/7 a	2/6 a,c	0/1
	H_2O	5/5 b,b,b,c,c	3/6 a,a,a	2/3 a,b	4/6 a,a,b,c
	MEA	5/5 b,b,b,b,c	5/6 a,b,b,b,c	2/2 b,c	1/2 c
	PAPP	5/5 c,c,c,c,d	4/6 a,b,c,c	2/3 a,a	1/2 b
	None	5/5 b,b,b,c,c	...	...	...

TABLE 4--Continued

Major Histopathologic Findings	Chemical Protectors	Proton-Radiation Doses			
		Group I 0 rad	Group II 939 rads	Group III 820 rads	Group IV 702 rads
Hemosiderosis	PG + H_2O	2/5 a,a	7/7 b,b,b,b,c,c,c	5/6 a,b,b,c,c	0/1
	H_2O	2/5 a,b	6/6 a,b,b,b,c,c	3/3 a,a,b	3/6 a,b,c
	MEA	4/5 a,a,a,b	6/6 a,b,b,b,b,c	2/2 a,b	2/2 a,b
	PAPP	5/5 a,a,a,a,b	5/6 a,b,b,c,c	3/3 a,b,b	1/2 a
	None	4/5 a,a,a,a	...	...	...
Hypocellularity of the bone marrow	PG + H_2O	0/5	7/7 c,c,c,c,c,c,d	5/5 c,c,c,d,d	1/1 c
	H_2O	0/5	6/6 b,c,c,c,d,d	3/3 c,c,c	4/6 b,b,b,c
	MEA	0/5	6/6 b,b,b,b,c,c	2/2 a,b	1/2 b
	PAPP	0/5	4/6 a,a,b,b	3/3 a,b,b	2/2 a,b
	None	0/5	...	...	...
Recovery of granulocytic elements in the bone marrow	PG + H_2O	0/5	0/5	0/5	1/1 c
	H_2O	0/5	0/5	0/3	3/6 b,c,c
	MEA	0/5	2/6 a,b	1/2 a	1/2 c
	PAPP	0/5	5/6 b,b,b	2/3 a,a	1/2 b
	None	0/5	...	...	...
Congestion of the bone marrow	PG + H_2O	0/5	7/7 a,,b,b,c,c,c,c	3/5 b,b,c	1/1 c
	H_2O	0/5	5/6 c,c,c,d,d	3/3 a,b,b	1/6 c
	MEA	0/5	5/6 b,b,c,c,c	2/2 a,b	1/2 a
	PAPP	0/5	3/6 a,b,b	1/2 a	1/2 b
	None	0/5	...	...	...
Atrophy of the thymus	PG + H_2O	0/5	4/4 a,a,c,c	4/4 b,b,c,c	1/1 b
	H_2O	0/5	5/5 b,b,b,c,c	2/2 b,c	6/6 a,a,a,a,b,b
	MEA	0/5	4/4 a,a,a,a	1/2 a	2/2 a,a
	PAPP	0/5	5/6 a,a,a,a,a	2/2 b,b	2/2 a,c
	None	0/5	...	...	...

TABLE 4--Continued

Major Histopathologic Findings	Chemical Protectors	Proton-Radiation Doses			
		Group I 0 rad	Group II 939 rads	Group III 820 rads	Group IV 702 rads
Fatty vacuolization of the cytoplasma of the hepatic cells	PG + H_2O	1/5 a	7/7 a,a,b,b,c,c,d	4/4 b,b,c,c	1/1 a
	H_2O	0/5	0/6 a,a,b,c,d	2/3 b,d	5/6 a,a,a,b,c,d
	MEA	0/5	6/6 a,b,c,c,d	0/2	2/2 a,b
	PAPP	0/5	5/6 a,a,b,b,d	2/2 a,d	2/2 a,b
	None	3/5 a,b,b	...	...	...
Irregularity in size of the hepatic cells nuclei	PG + H_2O	0/5	4/7 a,a,a,a	1/6 a	0/1
	H_2O	1/5	4/6 a,a,a,a	1/3 a	3/6 a,a,a
	MEA	0/5	4/6 a,a,a,b	2/2 a,a	1/6 b
	PAPP	0/5	1/6 c	0/3	2/2 a,b
	None	0/5	...	...	...
Focal hemorrhages and necrosis in the liver	PG + H_2O	0/5	0/7	0/6	1/1 a
	H_2O	0/5	1/6 b	1/3 a	0/6
	MEA	0/5	1/6 a	1/2 a	0/2
	PAPP	0/5	1/6 b	1/3 a	1/2 a
	None	0/5	...	...	...
Focal aspermatogenesis	PG + H_2O	0/5	2/7 a,a	2/6 a,c	1/1 b
	H_2O	0/5	2/6 a,b	0/3	4/6 a,c,c,d
	MEA	0/5	2/6 a,a	0/2	2/2 a,c
	PAPP	0/5	2/6 a,c	0/3	2/2 a,a
	None	0/5	...	...	...
Congestion and hemorrhages in the testis	PG + H_2O	0/5	1/7 b	1/6 c	0/1
	H_2O	0/5	1/6 a	0/3	1/6 a
	MEA	0/5	0/6	0/2	0/2
	PAPP	0/5	1/6 b	1/2 c	0/2
	None	0/5	...	...	...

TABLE 5

FREQUENCY AND SEVERITY OF MAJOR HISTOPATHOLOGIC FINDINGS IN X-IRRADIATED MICE

Major Histopathologic Findings	Chemical Protectors	X-Radiation Dose 666 rads
Spleen		
Atrophy of the lymphoid tissue	PG + H_2O	4/4 c,c,c,d
	H_2O	3/3 c,c,c
	MEA	1/2 b
	PAPP	2/2 a,b
Myeloblastic cell recovery particularly in the spleen	PG + H_2O	0/4
	H_2O	0/3
	MEA	2/2 c,c
	PAPP	2/2 b,c
Congestion of the spleen	PG + H_2O	3/4 b,b,c
	H_2O	2/3 b,b
	MEA	0/2
	PAPP	0/2
Bone marrow		
Hypocellularity	PG + H_2O	3/3 c,c,d
	H_2O	2/2 c,d
	MEA	0/2
	PAPP	1/2 a
Myeloid hyperplasia	PG + H_2O	0/3
	H_2O	0/2
	MEA	2/2 c,d
	PAPP	2/2 b,b
Congestion of the bone marrow	PG + H_2O	3/3 c,c,c
	H_2O	2/2 c,c
	MEA	1/2 b
	PAPP -	1/2 a
Spleen		
Extramedullary hematopoiesis	PG + H_2O	1/4 a
	H_2O	1/3 a
	MEA	2/2 b,c
	PAPP	2/2 c,c
Hemosiderosis	PG + H_2O	3/4 a,a,b
	H_2O	3/3 a,b,b
	MEA	0/2
	PAPP	0/2

TABLE 5—Continued

Major Histopathologic Findings	Chemical Protectors	X-Radiation Dose 666 rads
Thymus		
Atrophy of the thymus	PG + H_2O	2/4 a,c
	H_2O	1/1 b
	MEA	0/0
	PAPP	0/0
Liver		
Irregularity in size and shape of the nuclei	PG + H_2O	3/4 a,b,b
	H_2O	3/3 a,b,b
	MEA	2/2 a,a
	PAPP	2/3 b,c
Focal necrosis	PG + H_2O	2/4 b,c
	H_2O	1/3 b
	MEA	1/2 a
	PAPP	1/3 d
Focal hemorrhages in the liver	PG + H_2O	1/4 b
	H_2O	0/3
	MEA	1/2 a
	PAPP	0/3
Testis		
Focal aspermatogenesis	PG + H_2O	0/4
	H_2O	2/3 a,a
	MEA	2/2 a,b
	PAPP	3/3 a,b,d
Congestion	PG + H_2O	1/4 b
	H_2O	1/3 a
	MEA	0/2
	PAPP	0/3

radioprotective agents thus appeared to enhance extramedullary hematopoiesis in the x-rayed mice.

Bone marrow. All of the mice exposed to the proton radiation exhibited hypocellularity of moderate to very marked severity. The effect appeared to be directly related to the administered proton dose and was less severe in the mice pretreated with the radioprotective agents MEA or PAPP before the radiation exposure. In the bone marrow sections from the mice given 820 or 939 rads of proton radiation, the only cells which remained in several areas examined were the sinusoid lining cells, a few phagocytes and some quite abnormal megakariocytes. Other areas contained also a few plasma cells. Congestion of the bone marrow was another frequent finding in the proton-irradiated mice and the pre-irradiation administration of PAPP and MEA decreased the severity of this effect. The administration of the radioprotective agents appeared to stimulate recovery of the cells, particularly the myeloid elements. PAPP appeared to be somewhat more beneficial in this respect although there was little significant difference in the final histological effects in the animals given the two agents. Recovery was also observed in some of the animals given the lower dose (702 rads) of protons.

The x-rayed mice also exhibited hypocellularity and congestion of the bone marrow. These effects were less marked, however, in the mice pretreated with either MEA or PAPP. There was also enhanced recovery of the cells in the bone marrow sections from the MEA and PAPP-treated animals but contrary to the effects seen in the proton-irradiated animals, MEA appeared to be more effective than PAPP in promoting recovery in the x-rayed mice.

Thymus. Atrophy and congestion were frequent findings in the thymus of the proton irradiated mice. The radioprotective pretreatment partially prevented these changes and cellular recovery in this tissue appeared to be somewhat accelerated. Lymphadenitis was observed in one of the mice given 702 rads of proton radiation, and one other animal given this dose exhibited an abscess in the mediastinal fat. The histological effects of the proton irradiation in the mesenteric and mediastinal lymph nodes were similar to those seen in the thymus except that the lymphoid atrophy was often accompanied by severe sinusoidal dilatation and occasional hemorrhaging. These changes were also less severe in the animals given the MEA or PAPP pre-irradiation treatment.

Mild to moderate atrophy of the lymphoid tissue of the thymus and lymph nodes was also observed in the mice exposed to whole-body x-irradiation and these changes were again reduced in severity in the mice given MEA or PAPP prior to the x-ray exposure.

Testis. There was atrophy of the seminiferous tubules in the mice given 702 rads of proton radiation with or without the radioprotective treatment. Focal aspermatogenesis of some of the tubules was present in a few of the animals given 820 and 939 rads of proton radiation. One of the animals pretreated with PAPP which survived for 34 days after 939 rads of proton radiation exhibited a nearly complete aspermatogenesis. There was a marked decrease in the number of germinal cells and in some tubules only the Sertoli cells remained, while in others bizarre spermatogonia and spermatocytes together with a decreased number of sperm were present. Mitotic figures were absent in the cells of many tubules and markedly decreased in others. Most of the sections

from these animals exhibited a mixture of atrophic and active seminiferous tubules, occasionally containing bizarre multinucleated cells. Findings in the mice given 820 and 939 rads were similar to those receiving 702 rads, except for fewer numbers of mature spermatids and mature sperm. The least damage appeared to be in the testis of the mice given the protective agents prior to proton exposure. In addition to the tubular changes, there was also severe congestion of the interstitial tissue and hemorrhage involving the tunica albica.

Focal aspermatogenesis was observed in the testis of a few of the x-rayed mice and in all of the x-rayed animals which had been given either MEA or PAPP prior to the radiation exposure. The severity of the changes appeared to be greater in the protected group of mice. Congestion of the interstitial tissue was also noted in the x-rayed mice given 666 rads. The findings in these animals were generally similar to those seen in the mice given proton irradiation.

Liver. Most of the animals exposed to the proton radiation exhibited vacuolization of the cytoplasm of the hepatic cells in the periportal region. Similar changes were noted in a few of the control animals. The sections of the liver of mice which exhibited these changes stained positively for fat with Oil Red O. Another frequent finding in the proton-irradiated mice was mild to moderate irregularity of the hepatic cell nuclei. Both hyperchromic and pale nuclei were observed and there were scattered foci of necrotic cells with hemorrhage in sections from several of the proton-radiated mice. The hemorrhagic lesions were reminiscent of peliosis hepatis in humans in that there appeared to be dissolution of the liver framework with subsequent filling of the spaces with blood.

Vacuolization of the cytoplasm was seen in the hepatic cells of most of the mice exposed to the whole-body x-irradiation. These sections also stained positively for fat with Oil Red O. The size and shape of the nuclei varied considerably in the x-irradiated mice and there was moderate to marked focal necrosis in several of the liver sections. Massive focal hemorrhages similar to those seen in some of the sections from the proton-irradiated mice were observed in sections from one mouse which had been given 666 rads and in another given MEA prior to the x-ray exposure.

Heart. Some of the mice given 939 rads of proton radiation exhibited a brownish granular pigment in the atrioventricular valves, atrium and endocardium of the right ventricle and similar changes were also noted in the hearts of some of the animals which received the 820 rad exposure. Although this pigment appeared to be similar to hemosiderin in morphology, color, etc., it did not stain with Prussian Blue. There were subendocardial hemorrhages in a few of the animals given a proton dose of 939 rads, and one animal given 820 rads exhibited an inflammatory node in the pericardial fat.

Focal subendocardial hemorrhages and brownish pigment in the atrium and endocardium of the heart were seen in several of the x-irradiated animals in both the control and protected groups of mice.

Brain. Focal hemorrhages were observed in the cerebral and cerebellar gray matter of some of the proton-radiated animals. Hemorrhages confined to the subdural space were also noted in these animals. Only a few of the x-rayed mice exhibited hemorrhages in the brain, and all of these were subdural hemorrhages.

Lung. Peribronchial and perivascular accumulations of chronic inflammatory cells were present in mild to moderate degree in most of the control mice and in some of the proton-radiated animals. Acute and chronic bronchitis and pneumonitis were also observed in a few of the control and proton-irradiated animals at all dose levels. The severity of the lesions was greater in the irradiated animals, although the frequency of occurrence was smaller. Mild to very marked congestion was a rather frequent finding in the irradiated animals and was present at each dose level. It appeared to be somewhat less marked, however, in the mice pretreated with either PAPP or MEA prior to proton irradiation. Atelectasis, hemosiderosis, and edema were also observed in a few of the animals examined and did not appear to be related to either the dose of protons or the pre-irradiation treatment with the radioprotective agents.

Acute and chronic bronchitis and pneumonitis was observed in the x-rayed mice with or without the pre-irradiation protective treatment. Congestion was also a frequent finding in these animals and did not appear to be related to the administration of the protective agents.

Kidney. Perivascular accumulations of chronic inflammatory cells were observed in a few of the proton-irradiated animals. Extramedullary hematopoiesis was seen in one animal given 666 rads. Congestion was found in sections from a few of the animals and most of the mice exhibited some degree of terminal bacteremia.

Discussion

The gross and microscopic observations presented in this report were carried out in an effort to determine whether significant differences exist between the pathologic effects of whole-body exposure to high-energy proton radiation and those of x-radiation. Although relatively few animals were examined at each of the dosage levels included in these studies, it seems likely that differences in the pathologic effects of the two types of radiation are more quantitative than qualitative. The evaluation of the effects of the proton and x-ray exposures is complicated by the fact that in the present studies observations were made in terminal mice. Additional studies have been carried out in which the irradiated animals were sacrificed serially in time, and it is anticipated that examination of these mice will provide information concerning the pathogenesis of the lesions observed in the present studies.

The major effect of the acute proton exposure in the present studies was atrophy of the lymphoid tissue in the spleen, thymus, and lymph nodes; hypocellularity of bone marrow was also marked. Both of these effects were seen also in the x-rayed mice. Of particular interest was the finding that there was an enhanced hematopoietic recovery in the mice which were given radioprotective agents prior to either the proton or x-ray exposure. MEA and PAPP stimulated recovery of both the myeloblastic and erythroblastic elements, and, although the differences were not marked, PAPP appeared to be more effective in this respect in the proton-irradiated mice while MEA appeared to offer some advantage over PAPP in the x-rayed mice. These differences suggest that there may be significant differences in the ability of the two agents to protect against radiations of different LET values, and they tend to support the results of the mortality and longevity studies described elsewhere in this report (13).

Another major pathologic finding in the present studies was the atrophy of the seminiferous tubules in the irradiated animals. The injury of the seminiferous tubules in the proton-irradiated mice was more severe in the group given 702 rads than in the mice which received the higher doses. This unexpected finding is probably due in part to the differences in survival time and consequently in the time at which the mice were examined after the proton exposure. Although the existence of testis radiosensitivity has been known for almost 50 years (14), the detailed pathogenesis of this injury has been described only relatively recently (15,16,17). The studies of Heller (18) demonstrate that x-ray doses of 350 r result in loss of spermatogenesis by the ninth post-irradiation day and disappearance of spermatogonia by the end of the second post-irradiation week. In the present studies, there was only a slight reduction of spermatogonia and spermatocytes in both the proton and x-irradiated mice. The testicular atrophy was more marked, however, in the mice which had been given either of the radioprotective agents prior to the radiation exposure. However, there was little indication from the changes in the testis weight in the protected and non-protected mice that MEA or PAPP either prevented or enhanced the radiation injury. Previous studies by Kaplan and Lyon (19) and by Maisin et al. (20,21) have indicated that MEA does not protect germ cells against x-ray injury whereas those of Mendl (22) and Wang et al. (23) suggest that MEA inhibits the transient sterilizing effect of ionizing radiation.

The third major finding of the present studies was hemorrhage and congestion in the lungs, testis, brain, liver, various parts of the gastrointestinal tract, and elsewhere. The changes in the liver were of particular interest since the marginal hepatic cells were usually of normal appearance and thickness and there was no marked alteration of the architecture. The hemorrhagic pools seen in this tissue are similar to those seen in peliosis hepatis in humans for which inflammatory changes, liver cell necrosis, and a variety of other factors have been implicated in the pathogenesis (24-27). Kent and Thompson (28) have suggested that both general and local factors play a role, and that the development of the blood pools follows dilatation of certain groups of sinusoids.

The studies described in this report must be considered to be preliminary in nature because of the small number of animals examined, variation in time of examination after exposure, and the relatively small range of radiation dose levels considered. Additional experimental material from both the first and second proton studies is currently being processed, and the results of the examination of these tissues will be presented in subsequent reports.

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THE INFLUENCE OF EXPOSURE TO LOW LEVELS OF GAMMA OR FAST NEUTRON IRRADIATION ON THE LIFE SPAN OF ANIMALS

III. Studies on the Toxicity of Rare Earth Compounds and Their Influence on Radiation Lethality

David W. Bruce and Kenneth P. DuBois

This report concerns: Further studies on the acute intravenous toxicity of the rare earth compounds of the lanthanon series and in particular studies concerning the effects of praseodymium nitrate on intermediary metabolism when administered alone or in combination with whole body x-irradiation.

Immediate or ultimate application of the results: Because of the increase in the industrial utilization of the rare earth compounds, more information is needed on their toxicity. Equally important is the effect of simultaneous exposure to rare earth compounds and ionizing radiations that could result from a nuclear reactor accident. It is anticipated that this program will provide information on the toxicity and biological activity of these compounds and some of the problems that could arise from simultaneous exposure to radiation and fission products.

* * * * * * * * *

Studies on the acute intravenous toxicity of the rare earth nitrates (1,2) have shown they are highly toxic and that a sex difference exists with respect to the light lanthanons. The ionized salts of cerium, praseodymium, neodymium and samarium were found to be 7 to 10 times more toxic to female than to male rats. In addition, it was found that sublethal doses of whole body x-irradiation (50 r to 500 r) increased the number of observed mortalities 34% to 63% over the mortality resulting from the intravenous administration of 2 mgm./kgm. of praseodymium alone as the nitrate salt (3). In studies using each animal as its own control, it was found that intravenous administration of 2 mgm./kgm. and 4 mgm./kgm. of praseodymium caused a proportional decrease with respect to time in the blood glucose of female rats during the 12 to 48-hour period following administration. At any given time during this period the decrease in blood glucose after 4 mgm./kgm. was twice that seen after the administration of 2 mgm./kgm. X-irradiation (500 r) was found to augment the decrease in blood glucose 24 hours after 2 mgm./kgm. of praseodymium (3). Placing female rats on a high carbohydrate diet by the administration of a sucrose-saline solution ad libitum prior to the administration of praseodymium resulted in an apparent decrease in the toxicity of this compound. Daily administration of testosterone propionate (5 mgm./kgm.) for a period of 30 days prior to 2 mgm./kgm. of praseodymium also prevented or modified the resultant decrease in blood glucose normally seen after administration of this compound (3).

Materials and Methods. Adult, male and female Sprague-Dawley rats weighing 200 to 270 grams were employed for these experiments. The animals

were housed in air-conditioned quarters and given Rockland Rat Diet and water or specially prepared aqueous solutions containing 5% dextrose, 10% dextrose, 10% sucrose, or 10% fructose ad libitum. Unneutralized aqueous saline solutions of praseodymium nitrate were administered intravenously by tail vein; control animals received an equivalent volume of saline equal to 0.1% of the total body weight.

Blood glucose (total reducing value) was determined by the method of Folin and Malmros (4) employing the micromodifications of Park and Johnson (5). Serial samples of whole blood (0.05 ml. in duplicate) were obtained by sectioning the tail under local anesthesia. Glucose-6-phosphatase activity of the liver of male and female rats was determined by the method developed in this laboratory by DuBois et al. (6). The test system contained 0.15 ml. of barbital buffer (pH 7.4), 0.15 ml. of glucose-6-phosphate (10 mgm./ml.), 0.05 ml. of 0.04 M $MgCl_2$, 0.1 or 0.2 ml. of 2.5% liver homogenate and sufficient distilled water to make a final volume of 0.65 ml. The samples were incubated at 38° C. for 30 minutes and the reaction terminated by the addition of 0.1 ml. of 50% trichloroacetic acid. The activity was expressed as micrograms of phosphorus liberated per 5 mgm. of tissue per 30 minutes. Phosphorus was measured by the method of Fiske and Subbarow (7).

X-irradiation was administered as a single, total body exposure with a G. E. Maximar therapy unit. The radiation factors were as follows: 250 KVP, 15 ma., 0.25 mm. Cu and 1 mm. Al added filtration. The target-animal distance was 75 cm. and the dose rate was 35 r to 36 r/minute as measured in air with a Victoreen ionization chamber.

The nitrate compound used in this study was obtained from Lindsay Chemical Company, West Chicago, Illinois and the sodium salt of glucose-6-phosphate from the Nutritional Biochemical Company, Cleveland, Ohio.

Results

Effect of intravenous praseodymium nitrate on the blood glucose of male rats. Previous studies in this laboratory (2) demonstrated that intravenous administration of an approximate LD_{50} of praseodymium (2 mgm./kgm.) as the nitrate salt caused a marked decrease in the blood glucose of female rats but had no significant effect on the blood glucose of male rats. For this reason it was of interest to see whether a similar effect on blood glucose would be obtained if a dose of this rare earth metal approximating the LD_{50} 25 mgm./kgm.) was given to male rats.

Figure 1 shows the effect of 20 mgm./kgm. and 30 mgm./kgm. of praseodymium on the blood glucose of male rats. The values are plotted as per cent of the initial blood glucose values. Each animal served as its own control and each point on the curve represents the average values obtained for groups containing at least four animals. The control values in each rat were obtained 24 hours prior to praseodymium administration. Serial samples were also obtained from control animals at each 24-hour period.

In contrast to the decrease in blood glucose observed in female rats no appreciable change and perhaps a slight increase in blood glucose was observed in rats receiving 20 mgm./kgm. at the 72-hour test period. In spite

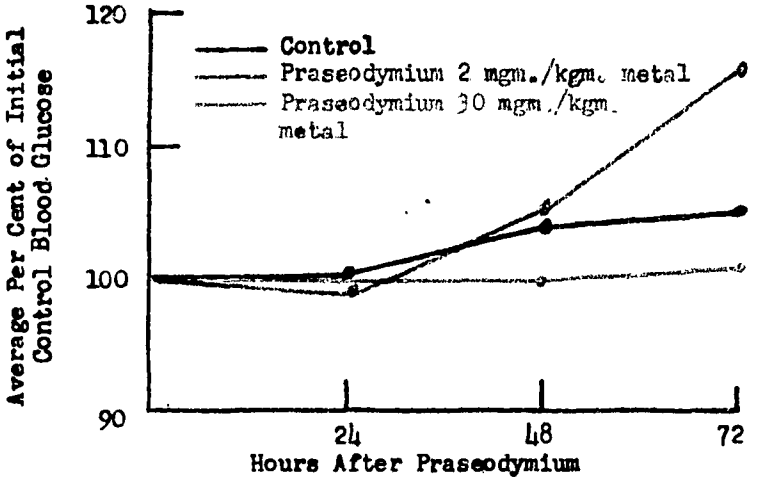

Figure 1. Effect of intravenous praseodymium nitrate on the blood glucose of male rats.

of the results obtained 20% of the animals given 20 mgm./kgm. and 30 mgm./kgm. succumbed during the first 72 hours while 40% of the total number of animals receiving 30 mgm./kgm. died between 72 and 120 hours post-injection.

Effect of intravenous praseodymium nitrate on the blood glucose of female rats receiving dextrose, sucrose, and fructose ad libitum. In previous studies (8) it was found that a 10% sucrose-saline solution administered ad libitum to female rats prior to the intravenous injection of praseodymium reduced the toxicity of the compound by approximately one-half. Because of the marked decrease in blood glucose observed after administration of 2 mgm./kgm. of praseodymium (6), these studies were conducted to determine the effect of ad libitum administration of sucrose, dextrose, and fructose on blood glucose. The aqueous solutions were given five days prior to the intravenous injection of 2 mgm./kgm. of praseodymium as the nitrate salt and continued throughout the test period; control blood samples and serial samples were obtained as previously described.

The effect of ad libitum administration of dextrose on blood glucose is shown in Figure 2. The values for control animals receiving either 5% or 10% dextrose or water are not significantly different from each other or from their initial control values. Although the amount of 5% dextrose consumed by each animal during any given 24-hour period ranged from 2 to 3 grams, the dietary modification did not change the blood glucose picture observed after administration of praseodymium alone. The average values were 77% and 41% of the initial control values at 24 and 48 hours respectively. Two of the four animals succumbed during the 48 to 72-hour period. In contrast, the four animals receiving the 10% dextrose solution survived the critical time period and no significant change from initial blood glucose control values was found at 24 hours, the average was 72% of the initial value at 72 hours. The average intake of 10% dextrose for each animal during each 24-hour period ranged from 6 to 9 grams.

In Figure 3 is shown the resultant blood glucose after administration of 10% fructose and 10% sucrose ad libitum. The variation in control values from each other and from initial control values is approximately the same as found in the control animals receiving dextrose. The administration of 10% fructose afforded no protection against the decrease in blood glucose caused by the administration of 2 mgm./kgm. of praseodymium. The blood glucose averaged 80% and 61% of the initial control values at 24 and 48 hours respectively. This is almost identical with the values of 82% and 59% obtained 24 and 48 hours, respectively, after praseodymium was given without dietary modification. As in the case of the 10% dextrose, the average intake of fructose for any 24-hour period was 6 to 9 grams for each animal. Two of the four animals died during the 48 to 72-hour period. Each of the animals that received the 10% sucrose consumed between 7 and 10 grams per day and all animals survived the test period. Twenty-four hours after praseodymium the blood glucose was 76% of the initial control value; however, it decreased only 5% to 71% of the initial control value at 48 hours. All of the sugar solutions seemed to enhance the recovery of the blood glucose of surviving animals.

Effect of intravenous praseodymium and whole body x-irradiation on the glucose-6-phosphatase activity of livers of male and female rats. In studies on the glucose-6-phosphatase activity of adult male and female rats, DuBois

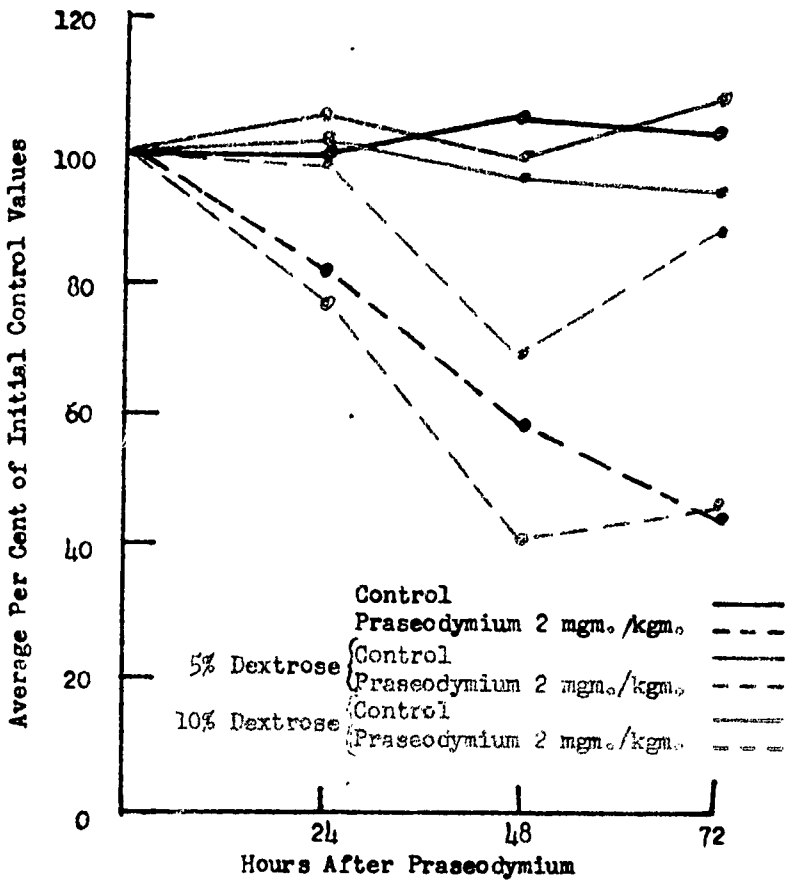

Figure 2. Effect of intravenously administered praseodymium on the blood glucose of female rats receiving dextrose ad libitum.

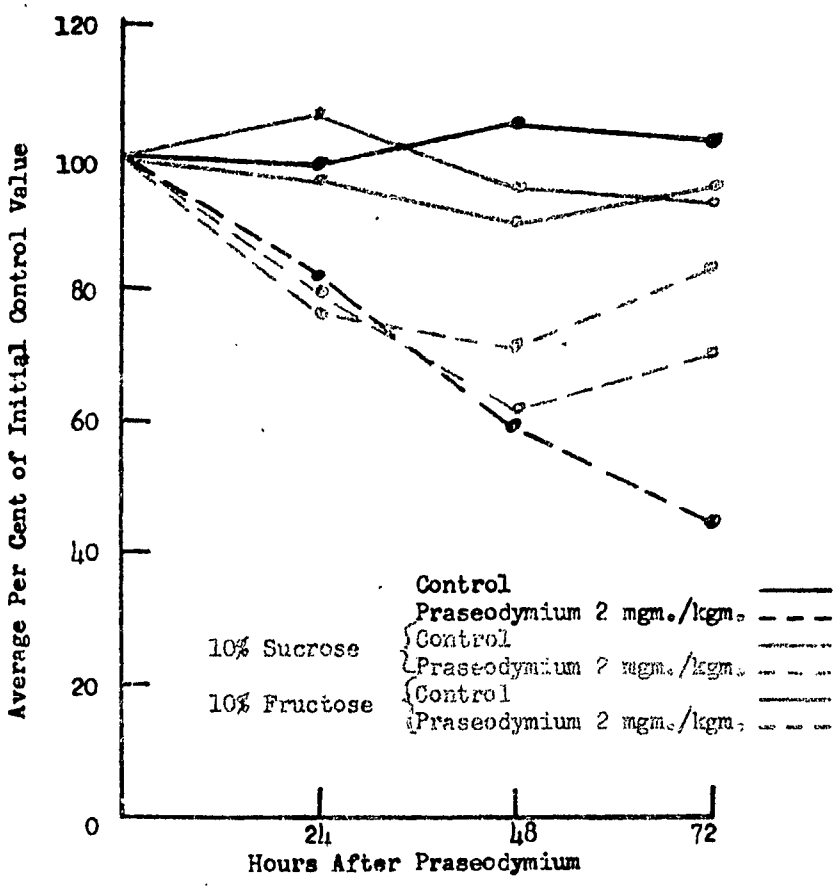

Figure 3. Effect of intravenously administered praseodymium nitrate on the blood glucose of female rats receiving sucrose and fructose ad libitum.

et al. (6) found the enzyme activity to be 29% lower in female rats. Similar results were obtained by Shull and Bautista (9) who found in addition that daily treatment of female rats with 10 mgm. of testosterone propionate for a period of two weeks increases the activity of the enzyme to almost the level of normal males. Previous studies in this laboratory (3) demonstrated that administration of testosterone propionate, 5 mgm./day for a period of 30 days, prior to the intravenous administration of 2 mgm./kgm. of praseodymium modified or prevented the decrease in blood glucose during the 24 to 72-hour period following praseodymium.

The results of administration of praseodymium and x-irradiation on the glucose-6-phosphatase are shown in Table 1. Duplicate assays were made with and without the addition of magnesium and the results are expressed as the mean plus or minus the standard deviation. The values obtained four hours after the intravenous administration of 4 mgm./kgm. of praseodymium or 24 hours after 500 r of x-ray do not differ significantly from the control values. An 18% to 25% decrease in glucose-6-phosphatase found 24 hours after praseodymium when given alone or in combination with x-ray may in part be attributed to the accumulation of fat in the liver.

The glucose-6-phosphatase activity of liver of male rats was reduced to the control level observed in females at 4 and 24 hours after intravenous administration of 20 mgm./kgm. of praseodymium. Whether or not this decrease is of any significance, studies in this report would suggest that it is not sufficient to have an effect on the blood glucose of male rats.

Influence of whole body radiation on rare earth toxicity. Continuing studies (3) on the influence of varying doses of whole body x-irradiation on mortality in female rats receiving praseodymium are shown in Table 3. Groups each containing 5 or 10 animals were given rare earth nitrate or x-ray alone or in combination. Praseodymium nitrate was given 10 to 15 minutes prior to whole body irradiation. No mortalities occurred among the irradiated controls given 500 r of x-ray nor among animals receiving 1 mgm./kgm. of praseodymium. One mgm./kgm. of praseodymium when given in combination with 500 r and 300 r of x-ray resulted in 33.3% and 20.0% mortality respectively. Further studies are being conducted to more fully evaluate the combined toxic effects of praseodymium nitrate and whole body radiation.

Discussion

The results of previous studies on the acute intravenous toxicity of praseodymium nitrate indicated that this compound is approximately ten times more toxic to female than to male rats (1). From our studies in female rats, it would seem that the magnitude of the observed decrease in blood glucose is sufficient to produce mortality. The protection afforded by the ad libitum administration of 10% dextrose and 10% sucrose tends to substantiate this conclusion. These studies also suggest that a diet high in carbohydrate is necessary to prevent mortality since the intake of 2 to 3 grams of dextrose did not prevent mortality. The failure of fructose to protect against mortality may be due to the difference in metabolism of this compound by the liver. Fructose conversion to fructose-1-phosphate and subsequently to dihydroxyacetone phosphate and glyceraldehyde and possibly glycerol might have

TABLE 1

EFFECTS OF INTRAVENOUS PRASEODYMIUM NITRATE AND WHOLE BODY X-IRRADIATION ON THE GLUCOSE-6-PHOSPHATASE ACTIVITY OF THE LIVERS OF FEMALE RATS[a]

Treatment	Hours After Treatment			
	4 Hours		24 Hours	
	No Mg	Mg	No Mg	Mg
Saline controls	24.6 ± 5.17 (8)[b]	30.2 ± 4.41 (8)		
Praseodymium nitrate 4 mgm./kgm. metal	24.7 ± 1.16 (4)	29.6 ± 2.15 (4)	20.2 ± 1.91 (8)	22.6 ± 1.51 (8)
Saline plus 500 r x-ray			24.1 ± 1.99 (4)	29.7 ± 2.46 (4)
Praseodymium nitrate 4 mgm./kgm. metal plus 500 r x-ray			19.1 ± 2.33 (4)	24.4 ± 2.52 (4)

[a]Each value given as the mean ± the standard deviation.

[b]Number of animals.

TABLE 2

EFFECT OF INTRAVENOUS PRASEODYMIUM NITRATE ON THE GLUCOSE-6-PHOSPHATASE ACTIVITY OF THE LIVER OF MALE RATS[a]

Treatment	Hours After Treatment			
	4 Hours		24 Hours	
	No Mg	Mg	No Mg	Mg
Saline controls	29.6 ± 5.05 (6)[b]	37.0 ± 3.66 (6)		
Praseodymium nitrate 20 mgm./kgm. metal	23.5 ± 1.82 (4)	30.8 ± 2.46 (4)	25.0 ± 2.33 (4)	31.7 ± 3.09 (4)

[a]Each value given as the mean ± the standard deviation.

[b]Number of animals.

TABLE 3

THE INFLUENCE OF VARYING DOSES OF WHOLE BODY X-IRRADIATION ON THE MORTALITY OF FEMALE RATS GIVEN PRASEODYMIUM NITRATE INTRAVENOUSLY

Treatment	Mortality[a]	% Mortality
Praseodymium nitrate 1 mgm./kgm. IV	0/10	0.0
Praseodymium nitrate 1 mgm./kgm. IV plus 500 r	5/15	33.3
Praseodymium nitrate 1 mgm./kgm. IV plus 300 r	2/10	20.0

[a]Mortality data based upon 10-day observation period.

an adverse effect on the toxicity of praseodymium. Studies by Snyder et al. (10) showed that the fatty infiltration caused by cerium consisted principally of neutral fat esters. The administration of an equitoxic dose of praseodymium to male rats had little effect on the blood glucose. The resistance of males to this biochemical change may be of significance in their lesser susceptibility to praseodymium.

The decrease in glucose-6-phosphatase activity in female rats at 24 hours after praseodymium alone or in combination with x-irradiation may be the result of accumulation of fat in the liver since no effect was observed four hours after administration, a time of maximum fixation of the light lanthanons by the liver (11).

Summary

1. Intravenous administration of 20 mgm./kgm. and 30 mgm./kgm. of praseodymium as the nitrate salt caused no significant decrease in the blood glucose of male rats. This is in contrast to the marked decrease in blood glucose in female rats caused by an equitoxic dose of this compound.

2. Evidence presented in this report indicates that dietary intake of 6 to 9 grams per day of sucrose or dextrose by female rats will prevent the mortality resulting from the intravenous administration of 2 mgm./kgm. of praseodymium as the nitrate salt. Dietary intake of 2 to 3 grams of dextrose or 6 to 9 grams of fructose per day was ineffective in preventing mortality. The results suggest that the mortalities are due to the decreased blood glucose.

3. Intravenous administration of 20 mgm./kgm. of praseodymium to male rats as the nitrate salt reduced the glucose-6-phosphatase activity of the liver 17% and 15% in 24 and 48 hours after administration, respectively. Four mgm./kgm. of praseodymium alone or in combination with 500 r of x-irradiation had no effect on the glucose-6-phosphatase activity of the liver of female rats four hours after administration. Fatty infiltration of the liver may account for the 18% to 25% decrease in activity found after 24 hours.

4. A 20% and 33% increase in mortality was observed when a sublethal dose of intravenous praseodymium (1 mgm./kgm.) was given 10 to 15 minutes prior to 300 r and 500 r of whole body x-irradiation.

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THE INFLUENCE OF EXPOSURE TO LOW LEVELS OF GAMMA AND FAST NEUTRON IRRADIATION ON THE LIFE SPAN OF ANIMALS

IV. Longevity in F1 Hybrid Offspring of Irradiated Mice

Ann M. Budy, John H. Rust, D. J. Mewissen, and Robert D. Boche

This report concerns: The longevity of the hybrid offspring from mice after irradiation of the male or female parents.

Immediate or ultimate application of the results: This study was initiated in this laboratory* several years ago to extend the available information on the effects of ionizing radiations on the offspring from irradiated animals. While the work was in progress, a preliminary communication (1) and several progress reports (2-6) were published. From the standpoint of the usefulness of the data obtained in this study, it seemed appropriate to present it in a concise form with appropriate statistical treatment of the data.

* * * * * * * * *

This experiment was designed to investigate the fate of hybrid offspring of an irradiated and a non-irradiated parent. The objective was to ascertain whether a single dose of whole-body x-irradiation delivered either to the male or female parent would be followed by a change in the life span of the progeny.

Since mutations induced by ionizing radiation are mostly deleterious and not completely recessive, it seemed feasible to determine their total additive effect by a comparative study of the relative fitness of successive F_1 litters as measured by the life span.

The progeny from a first mating of parents, prior to irradiation, served as the control group. Later progenies were obtained from successive matings of the same parents, after either the male or female was irradiated, and served as experimental groups.

Substantial indications of harmful influences are to be found in the work of early investigators. Timofeeff-Ressovsky (7) studied "Vitalitats-mutationen" from irradiated Drosophila and found this class of mutations to be very numerous compared to lethals and visibles. Hertwig (8) irradiated

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the testes of mice with x-rays and recorded the mortality for the pre- and post-sterility litters during the first 75 days after birth. She found an increased mortality during this interval for litters conceived before the onset of the induced sterility, but much less evidence of this in the later litters.

Stern and Novitski (9) have shown that many recessive lethals have some deleterious viability effects in heterozygous individuals. These viability phenomena in Drosophila have been studied further by Stern et al. (10), Moriwaki et al. (11), Burdick and Mukai (12), and Falk (13).

Muller (14,15) has estimated recessive detrimental mutational effects in Drosophila to be about 10% with 150 r exposure in the irradiated male. Foster et al. (16) in a study of the effect of whole-body x-irradiation of trout reported mortalities of 3.5% in controls as compared with 44.7% in fingerlings from parents receiving 1,000 r.

Russell (17) reported reduction of life expectancy of offspring conceived 19 to 23 days after exposure of male mice irradiated in a neutron flux. Recently Spalding and Strang (18) irradiated male mice in each of ten generations and found that mice from the irradiated germ line were approximately 24% less resistant to protracted gamma-ray stress than were controls. The data suggested that the inheritance of a genetic burden of recessive and/or sublethal mutants was detrimental to survival under conditions of extreme or abnormal environmental stress.

Materials and Methods. In an attempt to investigate possible differences in transmitting harmful effects, originating from either the male or female parent following x-irradiation, the present study was divided into two parts. The female parent was irradiated in one and the male parent in the other. The parents were males and females of pure inbred strains dba line 1 and C-57 Black subline 6 from Jackson Memorial Laboratory, Bar Harbor, Maine. They were four to five months old at the start of the experiment. Each pair of parents consisted of a male or female C-57 mouse and a dba mouse of the opposite sex. The C-57 parent, whether male or female, was the irradiated member in all groups. The two crosses are referred to as dba female X C-57 irradiated male and C-57 irradiated female X dba male.

The study was completed in two successive identically designed experiments, series A and series B, which were started one year apart. The purpose of the replicate (series B) was to increase the sample sizes for appropriate analysis.

In series A there were originally 14 dba female X C-57 irradiated male matings and 15 C-57 irradiated female X dba male matings. In series B there were 29 dba female X C-57 irradiated male matings, and 30 C-57 irradiated female X dba male matings. The parents were first mated prior to x-irradiation and the offspring of this mating, i.e., the weanlings, served as controls. In the second step of the experiment, at a 56-day interval after the first mating, the C-57 parents--males or females--were given 200 r of whole-body x-irradiation, using either a 250 KVP Kelekett or a General Electric Maximar X-ray machine. Physical factors were 1.0 mm. of Al and 0.5 mm. of Cu, 15 ma., 60 cm. source-target distance. The dose was delivered in

three minutes in series A and four minutes, twenty-four seconds in series B. The animals were exposed in groups of fifteen in perforated gelatin cells placed on a slowly revolving turntable. Following exposure, the C-57 mice were returned to their unexposed dba mates and allowed to breed for ten days. In subsequent steps of the study the same pairs, if still alive, were mated at 120 and 180 days after exposure. The total F_1 generation offspring of all these matings were kept for the duration of life in the same room, on the average of three animals per cage, under standard conditions of food, housing, and maintenance. Sexes were separated and litter mates were kept together for the duration of the experiment.

Results

Breeding. Breeding data are presented in Table 1. There were fewer pregnancies, in most instances, for each successive mating when the irradiated parent was the male. There were no significant differences between the controls and the 10-day mated irradiated females in the number of successful matings. Permanent sterility was induced in irradiated females within 120 days after x-irradiation. In groups where the male parent was irradiated, the litter sizes decreased sharply in the 180-day mating.

The sizes of the litters were not statistically different in the control and the 10-day offspring from irradiated males or females.

Mortality. The mortality data are presented in Table 2, in separate sets for series A and B. The groups of hybrids from matings between dba female and C-57 irradiated male at 180 days post-irradiation were of small size. Since variance analysis did not establish a significant difference in survival times between the 180-day and 120-day groups, it was decided to pool the progeny from both matings.

Cumulative survival curves are presented in Figures 1 and 2 for control, 10-day, and pooled 120 + 180-day offspring from an irradiated male, and control and 10-day offspring from an irradiated female, respectively. Corresponding groups from series A and B were pooled since variance analysis of life expectancies at successive 6-month intervals failed to reveal significant differences between such groups. Irradiation of the male parent appeared to reduce the life span of the offspring (Figure 1), as measured by the mean survival times. In contrast, in the offspring from an irradiated female there was no evidence of a decreased life expectany (Figure 2).

From the curves shown in Figure 1, it was difficult to decide whether the mortality pattern of the 10-day offspring was different from that of the 120 + 180-day offspring. Moreover, it seemed of interest to compare, on the basis of respective mortality rates, various groups of mice of either sex, as it is well known that female and male mice differ in this respect. Therefore, computation of gompertzian rates was carried out at successive 6-month intervals, using a method previously applied by Sacher (19).

In control groups of either sex, variance analysis of life expectancies at successive 6-month intervals showed no significant differences between groups originating from cross-matings of non-irradiated parents. Thus control offspring was pooled by sexes to increase sample sizes.

TABLE 1

BREEDING DATA

Groups[a]		Number of Pregnancies /Matings	Total Born	Average Litter	Total Weaned	Male/Female
Series A						
I	Control	13/14	71	5.5	52	31/21
	10 days	6/14	48	8.0	45	23/22
	120 days	3/11	16	5.3	11	8/3
	180 days	2/9	6	3.0	6	4/2
II	Control	15/15	119	7.9	117	47/70
	10 days	13/15	106	8.1	78	41/37
	120 days	0/13	...	...	...	.....
	180 days	0/13	...	...	...	.....
Series B						
I	Control	20/24	101	5.1	78	35/43
	10 days	15/24	66	4.4	61	29/32
	120 days	8/19	38	4.7	32	16/16
	180 days	7/18	22	3.1	22[b]	9/13[b]
II	Control	20/28	152	7.6	138	77/61
	10 days	23/26	178	7.9	166	74/91
	120 days	0/16	...	...	...	.....
	180 days	0/13	...	...	...	.....

[a]Group I = dba female X C-57 irradiated male. Group II = C-57 irradiated female X dba male.

[b]Two animals escaped after weaning and were discarded.

TABLE 2

MORTALITY DATA OF OFFSPRING FROM IRRADIATED AND NON-IRRADIATED PARENTS

Intervals (Months)		Series A				Series B			
		I		II		I		II	
		Male	Female	Male	Female	Male	Female	Male	Female
0-6	Control	0	0	1	0	0	3	3	1
	10 days	2	0	3	0	2	1	1	3
	120 + 180 days	4	0	..	..	2	0	..	..
6-12	Control	2	0	3	2	0	0	4	1
	10 days	3	1	6	1	2	1	0	6
	120 + 180 days	0	0	..	..	8	1	..	..
12-18	Control	4	10	6	7	14	6	28	5
	10 days	17	10	8	1	5	6	28	11
	120 + 180 days	0	2	..	..	14	15	..	..
18-24	Control	19	5	31	11	13	8	21	29
	10 days	1	6	19	5	19	12	37	26
	120 + 180 days	6	1	..	..	1	5	..	..
24-30	Control	5	6	5	30	8	24	21	21
	10 days	0	3	5	20	1	9	8	27
	120 + 180 days	2	2	..	..	0	6	..	..
30 and over	Control	1	0	1	20	0	2	0	4
	10 days	0	2	0	10	0	3	0	18
	120 + 180 days	0	0	..	..	0	0	..	..

Group I = dba female X C-57 irradiated male.
Group II = C-57 irradiated female X dba male.

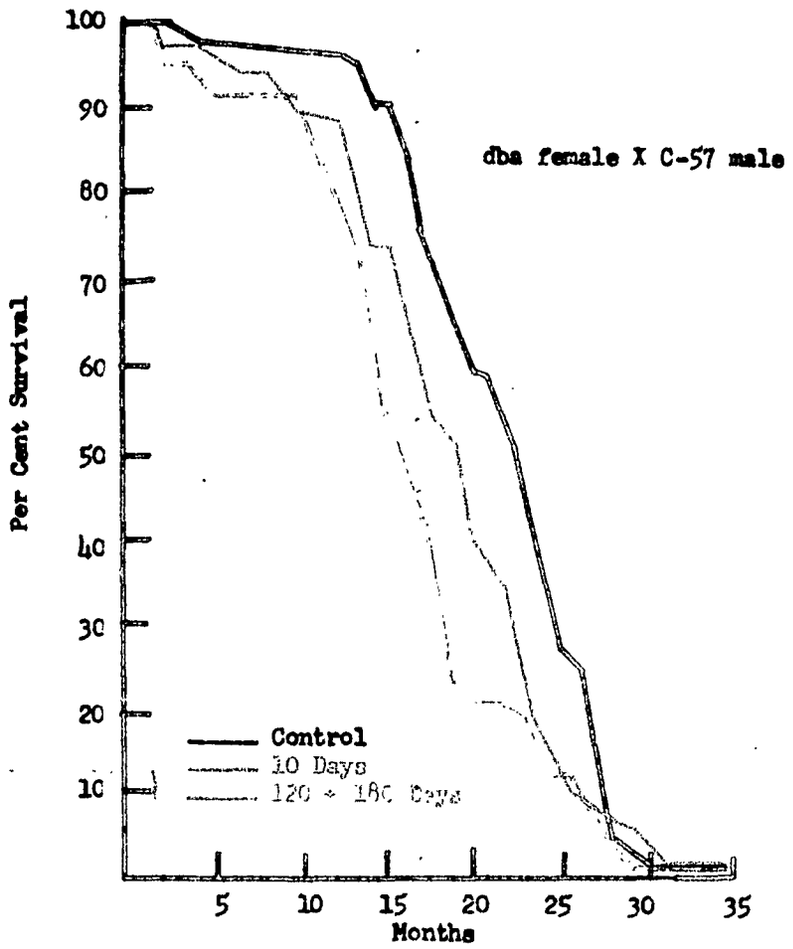

Figure 1. Survival of all offspring of control and irradiated male parents as a function of age in months.

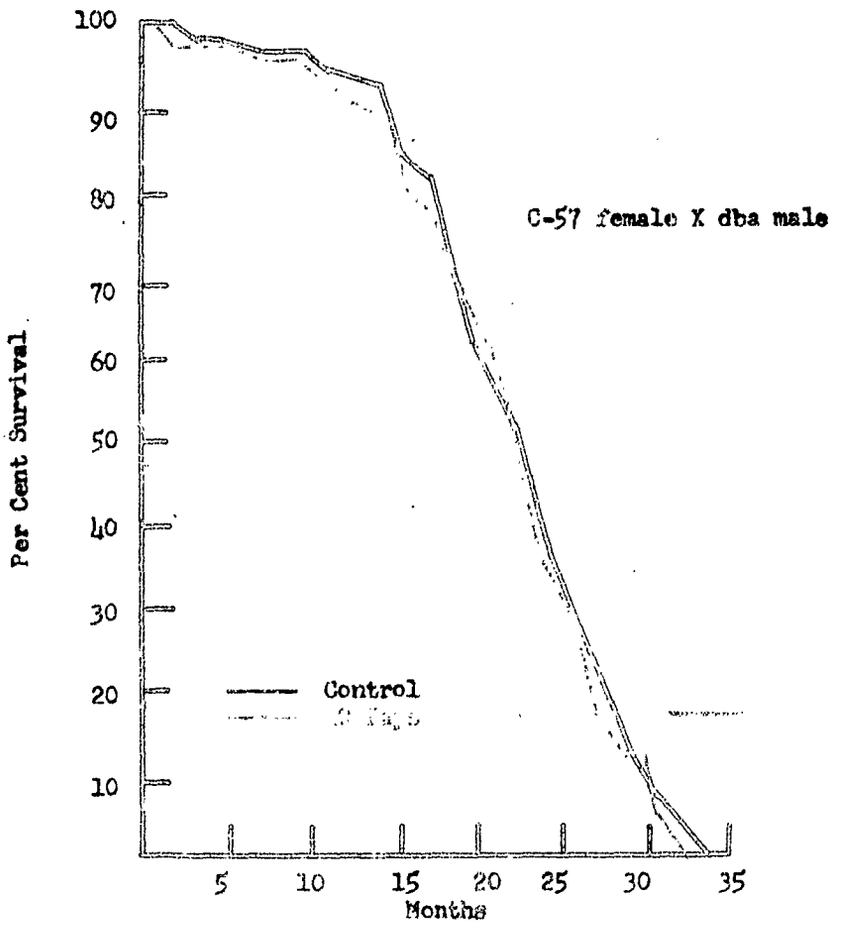

Figure 2. Survival of all offspring of control and irradiated female parents as a function of age in months.

In computing gompertzian mortality rates a weighting factor was used that was derived from the variances of proportion of mortality observed in each group. The rates were computed from:

$$\omega_t = \frac{1}{2h} \log \frac{N(t-h)}{N(t+h)}$$

where $2h$ is the selected time interval (six months) and $N(t)$ is the number of animals living at time t. Regression lines were fitted by the maximum likelihood method. Displacement of gompertzian intercepts was estimated from mean values calculated from the relevant regression equations. Whenever available data were inadequate for regression analysis of the computed mortality rates, the 95% fiducial limits of each representative point were computed. The results are given in Figures 3 and 4 and Tables 3 and 4.

Satisfactory regression lines of gompertzian mortality rates were obtained for both sexes from control groups and from 10-day offspring. The slopes of the Gompertz regressions did not differ significantly within groups of mice of the same sex but did differ between sexes. Moreover, there was a displacement of these Gompertz intercepts between sexes and between control progeny and 10-day offspring. These findings suggested that: (1) there was a specific age dependency of the mortality rate which appeared to be different in progeny of either sex, yet independent of the irradiation experience of the male parent, and (2) the displacement of the intercepts indicated that there was an observable reduction in life expectany in male and female progeny conceived by irradiated males mated within ten days after exposure.

The offspring pooled by sex from the matings at 120 and 180 days after the exposure of the male parent were too few to obtain a satisfactory regression. Therefore, the 95% fiducial limits were calculated for the relevant computed mortality rates. In groups of either sex the confidence limits for the separate representative points tended to embrace the regression line corresponding to the offspring conceived by males ten days after irradiation. It was concluded that the reduction in life span for 120- and 180-day post-irradiation progeny was probably equivalent to that observed for the 10-day post-irradiation offspring, irrespective of sex.

Correlation analysis were carried out on the data for 10-day offspring from C-57 irradiated female X dba male. They yielded regression equations whose linear coefficients were 0.157 for female offspring and 0.282 for male offspring (Table 3). These were divergent from the slopes previously estimated for the offspring of reciprocal matings. Therefore, it was believed that they did not provide an adequate basis for comparison, yet the shifts of the gompertzian rates, estimated at the 15-months survival time were consistent with previously computed intercepts (Table 4). Consequently the 95% fiducial limits of the computed mortality rates were also estimated. It may be seen (Figures 3 and 4) that the confidence limits tended to embrace the control regression lines, suggesting that irradiation of the female, in contrast to the male, did not affect the mortality rate of the progeny. All females were sterile at 120- and 180-day post-irradiation matings.

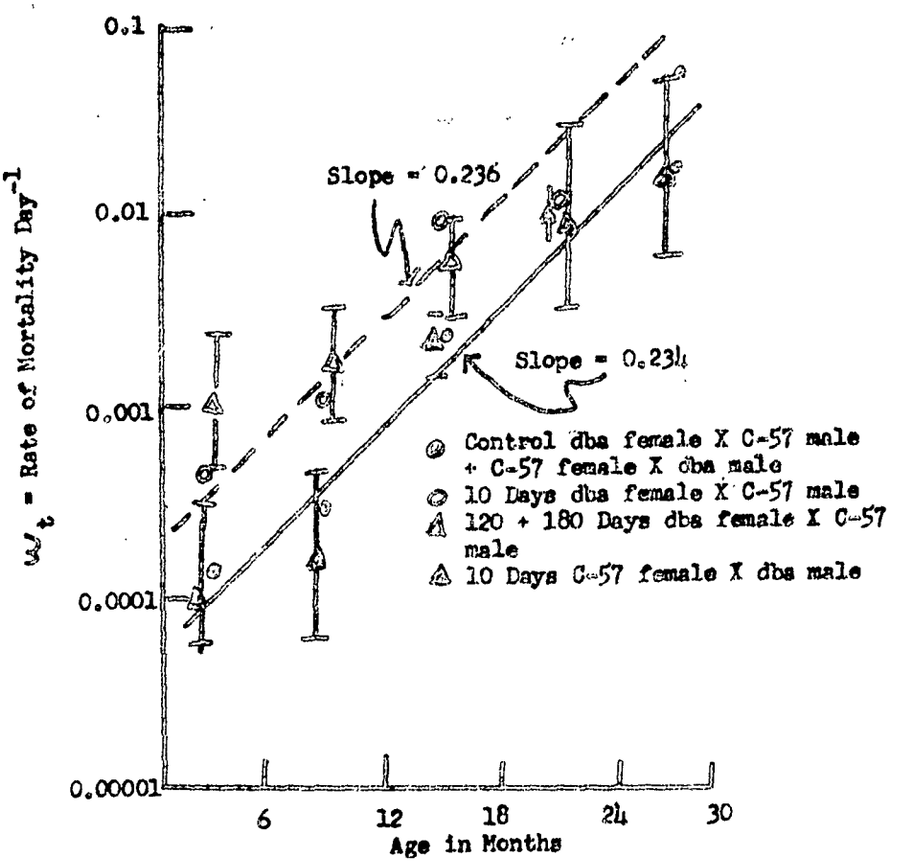

Figure 3. Gompertzian mortality rates as a function of age of male offspring of control and irradiated male and female parents. The C-57 parent is the irradiated member. The control includes all male offspring from non-irradiated parents.

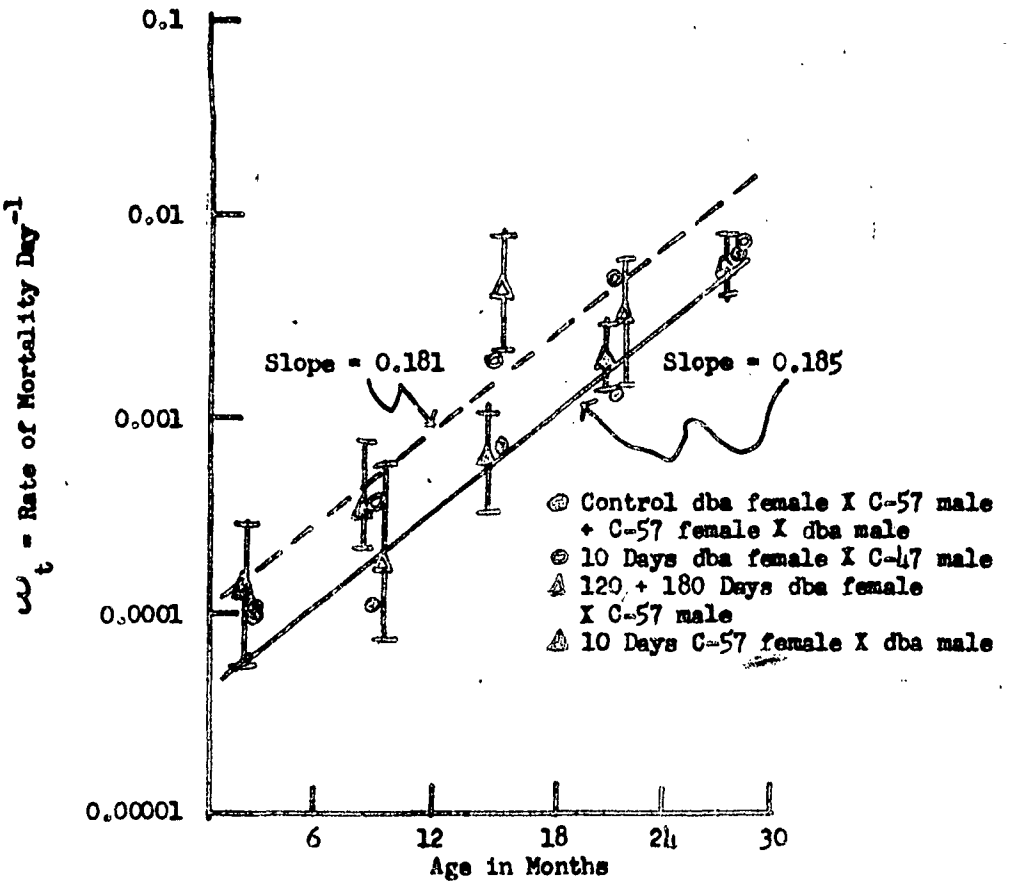

Figure 4. Gompertzian mortality rates as a function of age of female offspring of control and irradiated male and female parents. The C-57 parent is the irradiated member. The control includes all female offspring from non-irradiated parents.

TABLE 3

GOMPERTZIAN SLOPES AND FIDUCIAL LIMITS

Time of Conception	Hybrid Parents	Sex of Offspring	Slope	95 Per Cent Fiducial Limits
Control	dba female X C-57 male plus C-57 female X dba male	M	0.234	± 0.016
		F	0.185	± 0.018
10 days	I	M	0.236	± 0.050
		F	0.181	± 0.037
	II	M	0.282	± 0.025
		F	0.157	± 0.020
120 + 180 days	I	M	.....	.....
	II	F	.....	.....

Group I = dba female X C-57 irradiated male.
Group II = C-57 irradiated female X dba male.

TABLE 4

DISPLACEMENT OF GOMPERTZIAN RATES
(NATURAL LOG)

Groups Compared	Displacement
Control males and control females	1.08
Control males and Group I males (10 days)	1.26
Control females and Group I females (10 days)	0.88
Control males and Group II males (10 days)	0.05
Control females and Group II females (10 days)	0.23

Group I = dba female X C-57 irradiated male.
Group II = C-57 irradiated female X dba male.

Discussion

Our findings on sterility, fertility and litter sizes confirmed the work of Genther (20), Strandskov (21), Snell and Ames (22), Henson (23), and Amoroso and Parkes (24).

The general conclusion of the mortality data by graphical analysis as well as by computation of gompertzian rates was that differences could be noted between the groups from irradiated male parents but not from irradiated female parents.

Our findings with the progeny of irradiated male mice are similar to those obtained by Russell (17) who reported a reduced life span in the progeny of male mice mated 19 to 23 days after neutron irradiation. He suggested that the progeny in his study were derived from cells that were in a sensitive stage of gametogenesis at the time of irradiation. The same conclusions may be drawn from our study of the progeny conceived at 120 and 180 days after irradiation of the male parent. However, this cannot be said of the progeny from the irradiated male parent conceived within ten days following exposure. The progenies of this mating were born on an average of 22.3 days after irradiation of the male parent, and clearly indicates that these offspring were largely derived from irradiated spermatozoa. This suggests that spermatozoa were also sensitive to radiation as were the cells under consideration in Russell's study, and in the male gametes involved in late matings in our study.

The reduced life span exhibited by the progeny from the 120- and 180-day matings of the dba female X C-57 irradiated male as compared with those of the 10-day matings, was contrary to expectation since Hertwig (8) observed diminished genetic effects in progeny conceived in the post-sterility period. Russell (17) also suggested that such effects in late litters might be diminished. Our results suggested that spermatogonia might carry the life span reducing injury for several cell generations. In fact, the reduced life span of the progeny might well be a permanent genetic burden for male gametes.

The absence of shortening of life span in the progeny of irradiated females was also unexpected. Russell et al. (25) showed that mutations at specific loci were increased, regardless of the sex of the irradiated parent; they concluded that for induced mutations at specific loci, acute irradiation of oocytes was at least as effective as acute irradiation of spermatogonia. Our data did not support this view, but rather tended to agree with the conclusion made by Carter (26) that female germinal cells are less sensitive to radiation than those of the male. However, such a comparison with our results is pertinent only to the extent that one can reasonably assume that the reduction in longevity as observed in hybrid mice from an irradiated parent is related to the same mutational processes studied by Russel et al. (25) and Carter (26).

Our study yielded no clues as to why a reduction of life expectancy was observed in the progeny from irradiated male, but not from irradiated females.

Summary

This experiment was designed in an attempt to study the longevity of offspring from an irradiated and a non-irradiated parent. Mice of strains dba and C-57 Black were mated and cross-mated 56 days before and 10, 120, and 180 days after 200 r whole-body x-irradiation was delivered either to the male or female parent.

There was a reduction in the number of successful pregnancies and in the litter size of the 120-day and 180-day matings with the irradiated male parent. This reduction in pregnancies did not hold for the irradiated female parent in the 10-day mating; they were sterile in subsequent matings. The percentage of animals born and living until weaned and the distribution of sexes at weaning time did not follow a consistent pattern.

Irradiation of the male parent reduced the life span of its progeny without regard to their sex whether conceived immediately after irradiation or 120 or 180 days later. It is likely that spermatozoa were irradiated in the first matings and spermatogonia in the second and third.

Irradiation of the female parent did not reduce the life span of its progeny, regardless of sex. Females given 200 r whole-body x-irradiation were sterile at 120 and 180 days after exposure.

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